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THE CHEMICAL IDENTIFICATION OF GENE-CONTROLLED PIGMENTS IN PLATYPOECILUS AND XIPHOPHORUS AND COMPARISONS WITH OTHER TROPICAL FISH[1]

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THIS paper presents a study of the pigments and cells that produce the red, yellow, and orange colors in certain fish with special reference to those cases where genetic analyses have been made of color inheritance. The following is the list of species which we have studied: *Platypoecilus maculatus* and *Xiphophorus helleri* (the swordtail) of the family Poecilidae, *Oryzias* (*Aplocheilus*) *latipes* (the Medaka) of the family Cyprinodontidae, and four species of the Family Osphronemidae: *Macropodus opercularis* L. (the paradise fish), *Colisa lalia* (dwarf gourami), *Colisa fasciata* (striped gourami), and *Betta splendens* (Siamese fighting fish). The classification is summarized in table 3.

All pigments studied are intracellular, being carried by chromatophores which are located in the dermis. The types of chromatophores with which we are especially concerned are the yellow cells, or xanthophores, the red cells, or erythrophores, and a type designated as the xantho-erythrophores (fig. 1) which contain two sharply distinguishable pigments. The xanthophores and erythrophores integrade in species of the family Osphronemidae so that cells of intermediate color may be seen. In these cases, however, the color is uniform throughout the cell, making them quite different from the xantho-erythrophores.

PLATYPOECILUS AND XIPHOPHORUS

These two closely related Mexican fish have been the subject of extensive genetic studies by various authors, especially by BELLAMY, GORDON, KOSSWIG, and BREIDER. GORDON (1931, 1937) may be consulted for the bibliography of the genetics of these fish.

Our studies indicate that the pigments zeaxanthin and lutein produce the yellow color in these fish and that the red is due to erythopterin. BELLAMY (1924, 1928, 1933), FRASER and GORDON (1929), and GORDON (1937a) find that in Platypoecilus the factor for red *R* is sex-linked and the female is the heterogametic sex. Red crossed with gold (FRASER and GORDON 1929) gives typical sex-linked ratios of ZW type with red dominant to gold. The situation for our purposes, however, is more accurately stated

[1] This investigation, including the preparation of the colored plate, was made possible by grants from the Atwater Fund and from the Denison Research Fund of WESLEYAN UNIVERSITY.

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by considering the above as a cross between red and non-red with red dominant over non-red. Similarly, crosses between the autosomal character stippled and gold (stippled is regarded as dominant to gold) is best expressed as a cross between stippled and non-stippled. In these two cases, the stippled and red factors merely conceal the yellow to a certain extent and may therefore be considered to be partially epistatic to the yellow color. GORDON (1927) indicates in his table (page 261) that xanthophores were present in all types, and BELLAMY (1933) on page 523 refers to the typical effects of gene *g* in various combinations. Both these statements we understand to be a recognition of the presence of the yellow pigments in various types other than that known by the fanciers as gold.

Our observations confirm the presence of the yellow pigment in all varieties of Platypoecilus that we have studied. However, while in all non-red types it is the exclusive color of the xanthophores, in the red form the yellow and red pigments are contained in the same type of cell which is called a xantho-erythrophore. Therefore, the presence of the red producing gene brings about the added development of the red pigment in cells which we consider to be homologous to the xanthophore of other types. In the xanthophores, the yellow appears to be present in two forms—as globules about 1 micron in diameter and in solution in the cytoplasm. When cells disintegrate, the globules appear to coalesce. Application of a gentle heat by warming the microscopic mount appears to hasten the process. The xantho-erythrophores are characterized by dense yellow pigment at the center and a red pigment at the periphery of the main body of the cell, but yellow pigment may be diffused in the cytoplasm (Plate 1). This plate is from a natural color photograph taken on a scale immediately after removal from the living fish. It therefore shows an optical section at some depth in the fresh tissue. Observations with reflected light show extraordinarily delicate cell processes of a yellow color which are too minute to contain globules. The red pigment is in the form of granules which may collect on the surface of the droplets of yellow pigment or be free in the cytoplasm. These granules do not coalesce under any conditions observed under the microscope. The chemical nature and methods of identification of the pigments are outlined later in this paper.

The genetics of *Xiphophorus helleri* has not as yet been so extensively studied as in Platypoecilus. The two species may be crossed in the laboratory (GORDON 1931b). In some cases when the gene *Sp* (spotted) is present, this cross produces the well known melanotic hybrids. Genetic studies have been made by KERRIGAN (1934), BREIDER (1935), KOSSWIG (1935), GORDON, (1937b, 1940). In Xiphophorus we are again concerned with the red and yellow pigments. There is some reason for believing that the red

DESCRIPTION OF PLATE I

FIGURE 1.—A xantho-erythrophore from *Xiphophorus helleri*. Photograph (Kodachrome) of living cell taken with oil immersion. Magnification ×760 Photograph was taken by MARIAN HEDENBERG.

pigment in certain varieties of Xiphophorus has been introduced by hybridization with Platypoecilus (GORDON 1931, INNES 1935) and that different red types may have had separate origin, one from the original red (Rubra, *R*) in Platypoecilus and another from the red finned yellow platy (gene *Dr*, formerly called *Rf*). It has been suggested that the gene *R* from Platypoecilus spreads the red color over the whole body of the hybrid, while the gene *Dr*, similarly introduced, does not color the ventral throat region (GORDON 1931a). This strain, according to INNES (1935, page 323) arose in 1930. Our material shows this same white throat and quite possibly originates from this same stock. KOSSWIG (1935) uses the symbol *Rb* for the red color. Since there is at present no agreement as to symbolism, we

TABLE 1

Green ("wild")	*rr*	*StSt*	*PP*
Brick red	*RR*	*StSt*	*PP*
Gold	*rr*	*stst*	*PP*
"Red Albino"	*RR*	*StSt*	*pp*
Albino	*rr*	*StSt*	*pp*

Genes *R*=red (may be *Dr* of GORDON), *r*=absence of red; *St*=stippled, *st*=absence of stipple; *P*=pigment factor necessary for development of melanin, *p* inhibits melanin production. Melanophores appear only when genes *St* and *P* both are present.

are using *R* to designate the factor for red in the type we have used. Whatever may have been the origin, the genetic situation appears in many respects similar to that in Platypoecilus.

The wild type green (homologous to stippled in Platypoecilus), when crossed with the gold type, gives an F_2 ratio of 3 green (stippled) to 1 gold (non-stippled) (GORDON 1937b). Here, as in Platypoecilus, xanthophores are present in both types but are not noticeable except in the gold form, so that we may consider the stippled (green) to be epistatic to yellow. Similarly (GORDON 1940), the factor for red need not be regarded as an allele of yellow but as an allele of non-red and as partially epistatic to yellow, because the red pigment of the erythro-xanthophores makes the presence of yellow less obvious. GORDON's and BREIDER's work indicates the genetic formulae given in table 1.

All of the five types listed in the table carry yellow pigment. In the brick red and "red albino" it is in the xantho-erythrophore, and in the albino the xanthophores are reduced in size. The "red albino" has a red body color with little or no melanin and has the red eyes of a typical albino. This type was reported arising in a cross described by KOSSWIG (1935), and his results have been duplicated in our laboratory. Of the types listed, brick red and gold (these are both names given by fish fanciers) were the forms used for our chemical analyses of pigments.

ORYZIAS

The genetics of *Oryzias* (*Haplocheilus*) *latipes*, a Japanese fish, commonly known as the Medaka, have been studied by AIDA (1921, 1930, 1936). Colors are produced by melanophores and xanthophores. Types carrying gene *B* owe their dark color to fully developed melanophores, and those having the recessive *b* have melanophores with very little melanin (GOODRICH 1927); this deficiency is interpreted by GOODRICH (1933) to be due to the absence of chromogen, since the 'dopa' reaction indicates the presence of the necessary oxydase. Types carrying gene *R* are yellow due to the well developed xanthophores, and those having the recessive *r* have homologous cells with little or no pigment (GOODRICH 1927). Our chemical analyses show the yellow pigment to be lutein.

OSPHRONEMIDAE

The other fish examined are from genera of a single family of the order Labyrinthici. By various authors they are placed in the family Anabantidae (*cf.* WEBER and DE BEAUFORT 1922), but by JORDAN (1923) they are placed in the more restricted family Osphronemidae. The species studies are found in Southeast Asia. For colored figures see INNES (1935).

Macropodus opercularis has long been known as an aquarium fish. KOSSWIG (1935) and GOODRICH and SMITH (1937) have shown that the dark form is a simple Mendelian dominant to the albino and that the albino completely lacks the melanophores. The red or yellow cells, however, are present in both types. These cells are very similar to each other, and the distinction is apparently due to slight differences in the color of the pigment and not to the presence of two physically different pigments as in Platypoecilus and Xiphophorus. The pigment appears to be in minute droplets, but as in the case of the yellow pigment in the two previously discussed types, these globules may coalesce on disintegration of the cell or when slightly warmed. Our analyses show the presence of the two xanthophylls, violaxanthin and lutein. When these pigments were obtained in solution in our experiments, the solution of violaxanthin was of a slightly more orange color than that of lutein. A preponderance of violaxanthin in certain cells may cause their more reddish color.

Colisa lalia and *Colisa fasciata* were formerly placed in the genus Trichogaster. These two types have markings very similar to those of Macropodus. Our studies show the presence of the same pigments, violaxanthin and lutein.

Betta splendens Regan (the Siamese fighting fish) exhibits a great variety of types, and no satisfactory genetic analysis have yet been made. GOODRICH and MERCER (1934) showed that the dark types are dominant to the light forms (known to fanciers as Betta cambodia and by various trade

names). Red and yellow pigments are present in both varieties, but in "cambodia" the red tends to be largely restricted to the fins. Our analyses shows the red to be erythropterin and the yellow to be lutein.

CHEMISTRY OF THE PIGMENTS

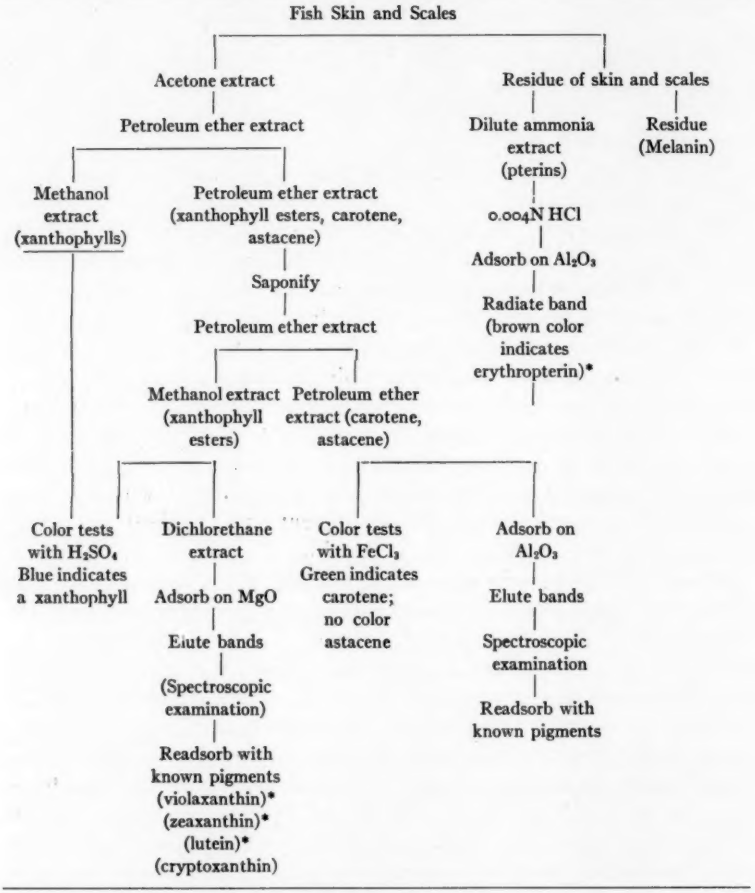
The material for these studies was obtained from laboratory stocks originally purchased from fish fanciers, mostly from M. MATSUNO of Flushing, New York. The chemical studies were made by M. S. ARRICK in consultation with DR. G. A. HILL.

The procedure, which is outlined in table 2, is based on methods employed by SUMMER and FOX (1933, 1935), SCHOPF and BECKER (1933 and 1936), STRAIN (1938), and KUHN and BROCKMANN (1932). The brain of the fish was first pithed and the animal then dipped in boiling water for about 20 seconds. STRAIN (1938) found that the oxidation of carotenoid is greatly reduced if the material is put in water of 90–100°C for a short time. The skin and scales were then ground in sand with acetone. The acetone extracted the carotenoid pigments, while melanin and the red pigment later shown to be erythropterin remained in the residue. The acetone soluble pigments were transferred from acetone to petroleum ether by the addition of 10 cc of petroleum ether and water saturated with NaCl. The supernatant petroleum ether was separated and mixed with an equal quantity of absolute methanol. When sufficient water to dilute the alcohol to 80–90 percent was added, the methanol layer separated. The pigments remained in the petroleum ether layer, indicating that they must be carotenes, xanthophyll esters, or astacene, whereas pigments remaining in the methanol would have been xanthophyll. This indicates that the pigments exist as esters in the tissues. The petroleum ether solution was saponified by adding an equal volume of C_2H_5OH-KOH (5 percent) and warming for two hours at 40–50°C. This saponification converts xanthophyll esters into xanthophylls. After saponification, the pigments were extracted with petroleum ether by addition of water saturated with NaCl. Care has to be taken to prevent formation of colloidal solutions at this stage. The petroleum ether extract was again mixed with absolute methanol, and when water was added, two phases formed, and pigment passed into the hypophasic methanol layer. This indicated that the pigments were of the xanthophyll group. If pigment had passed into the petroleum ether layer at this stage, it would be suspected of being a carotene and would be tested by an adsorption method.

The separation of the xanthophyll pigments was accomplished by means of the micro-chromatographic adsorption method. The pigments were transferred to dichloroethane by addition of water saturated with NaCl. An adsorption column three mm in diameter was prepared from a suspen-

TABLE 2

Outline of procedure based on methods of SUMNER *and* FOX *(1933, 1935)*, STRAIN *(1938)*, KUHN *and* BROCKMANN *(1932)*



* Indicates pigments obtained in this investigation.

sion of a 1:2 mixture of magnesium oxide and Hyflo super-cel[2] in dichloroethane. When the pigment solution was poured on the adsorbent, a wide orange-red band formed at the top of the column. As this was further washed with dichloroethane, bands of pigment separated. Previously

[2] Hyflo super-cel F. A. 501, manufactured by Johns-Manville, is a heat treated siliceous earth for diluting the absorbent.

xanthophyll crystals had been obtained from Eimer and Amend specified as having been extracted from alfalfa. These were tested by the adsorption method, and four bands were obtained. Pigments separated from these bands were examined to obtain the adsorption maxima with a Zeiss "pocket" spectroscope. These gave adsorption maxima corresponding with those given by STRAIN (1938) for violaxanthin, zeaxanthin, lutein, and cryptoxanthin, respectively.

Confirmatory evidence that these were the pigments concerned was obtained from the order of adsorption on the chromatogram which was to order named above with the first, violaxanthin, being uppermost. This order and the colors corresponded with that reported by STRAIN. Also, the addition of concentrated H_2SO_4 to a dichloroethane solution of the pigment from band 2 gave a blue color for the acid and dichloroethane layer, which is a test for zeaxanthin (STRAIN 1938). Similar treatment of band 3 yielded blue color only in the acid layer, suggesting lutein (STRAIN 1938). When an ethereal solution of band 1 was treated with HCl, a light blue color appeared in the acid layer—a test for violaxanthin (FOX 1936).

The bands obtained from the fish extracts were separated and eluted with ethyl alcohol and transferred to dichloroethane. These separate solutions were then mixed respectively with the separate solution of the known individual alfalfa pigments and again readsorbed on separate columns. When only one band formed, the pigments were judged to be identical.

The red pigment of the erythrophores was extracted by grinding skin and scales with dilute ammonium hydroxide. It had been found very resistant to the solvent action of acetone, methyl alcohol, ethyl alcohol, petroleum ether, acetic acid, formalin, and hexane and had not been removed by boiling. The melanin (soluble in concentrated NH_4OH) remained in the residue. The deep red extract changed to yellow color upon acidification with HCl. These properties suggested a pterin pigment. A 0.004N HCl solution of the pigment was subjected to chromatographic adsorption on aluminum oxide and Hyflo super-cel (1–2) column. A single orange band formed, strongly adsorbed at the top of the column. Radiation of the band with mercury vapor quartz lamp changed it to a light brown color. This indicated that the pigment was probably erythropterin ($C_{19}H_{17-18}(O+H)_{20-21}$).

The above procedure was applied to the various species the following results:

Platypoecilus maculatus: Two varieties of this species (the names are those given by fanciers) were studied. The blood-red "platy" has xanthoerythrophores, and the analysis indicated that the red was erythropterin, and the yellow was due to zeaxanthin and lutein. The Golden Crescent Moon has a gold body color with a black band across the caudal peduncle and a red dorsal fin. The gold pigments were zeaxanthin and lutein, and the

red pigment of the dorsal fin was erythropterin. *Xiphophorus helleri:* "Blood-red" and "brick-red" varieties both have the xantho-erythrophores. The pigments were the same as in Platypoecilus. The red was erythropterin and the yellow zeaxanthin and lutein. The golden swordtail, which also has a red stripe, carried the same pigments. *Oryzias (haplocheilus) latipes:* the yellow type was used and the only pigment other than melanin was lutein. *Colisa lalia, Colisa fasciata,* and *Macropodus opercularis* all showed the presence of violaxanthin and lutein. *Betta splendens:* the yellow was lutein and the red erythropterin. A deep red variety and a yellow bodied type known to fanciers as the red betta and Betta cambodia, respectively, were utilized.

THE NON-MELANIN PIGMENTS OF FISH

In this paper we are chiefly concerned with pigments of the skin other than melanin. KRUKENBERG (1882), by the limited chemical and physical methods then available, described pigments from *Carassius auratus, Cyprinus carpio,* and *Muraena helena.* These were classified as lipochromes, a term since superseded. CUNNINGHAM and MAC MUNN (1893), working on the flat fishes, noticed a similarity of lipochromes in related fish. Since this early period there has been an extensive development of carotenoid chemistry, but this has included very little work on fish. LEDERER (1935) isolated astacene from *Carassius auratus* and *Bery decadactylus.* In Carassius he also discovered small quantities of carotene and xanthophyll. These results, in the course of work preparatory for this investigation, have been confirmed in our laboratory. LEDERER (1935) also found that carotene is present in *Pleuronectus flesus,* xanthophyll in *Scomber scombus,* while both are present in *Cottus bubalis* and *Herophis aequoreus.* FOX (1936) finds a pigment identical or isomeric with taraxanthin in two Pacific fish, the *Fundulus parvipinnus* and *Hypsypops rubicunda.* Pigments have been identified in other parts of fish, as in the muscle of salmon (EULER et al. 1933), in the eyes (LONNBERG 1937), and in various marine animal oils (BURKHARDT et al. 1934).

The blues, greens, purples, and silver white of many fish is due in part or wholly to reflection and diffraction and refraction of light from guanine crystals. (See discussion by RAUTHER 1927.) Blue pigment, however, has been described in Australian parrot fish of the genus Odax by FRANCIS (1875) who made some chemical studies. GOODRICH and HEDENBURG (1941) have noted a similar pigment in Bermuda parrot fish, and ZEYNECK (1901, 1902, 1912) considers the blue pigment in a Mediterranean wrasse *Crenilabrus parvo* to be a carotenoid albumen.

Pigments which upon further investigation we think may prove to be pterins have been described in numbers of fish. BALLOWITZ (1913) found

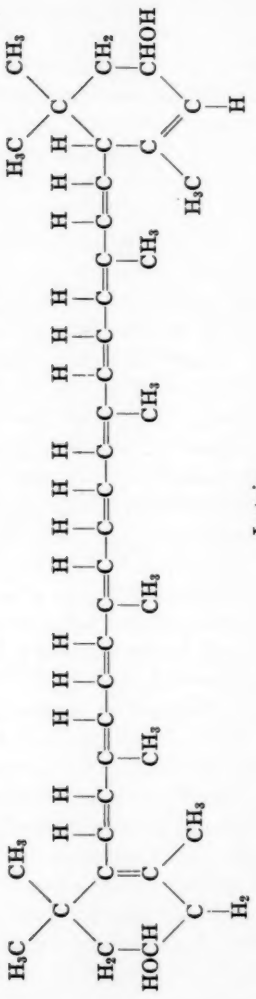
in certain fresh water fish (*Fundulus chaperi, Haplocheilus chaperi, Pantodon bucholsi*) a red pigment not soluble in alcohol or ether; he reported also the brown-red alcohol resisting cells in Xiphophorous and *Betta rubra*. BECHER (1924) found similar cells in Essox. KOSSWIG (1935) noted the presence of these relatively insoluble pigments in Platypoecilus and Xiphophorus and suggested that they might belong in the melanin series, but he reports no chemical investigation. A similar suggestion is made by BREIDER (1936). We do not know of any previous descriptions of a pterin from the integuments of fish. They have been found most abundantly in insects and among the metabolic products of vertebrates. (See review by LEDERER 1940.)

As noted above, it has been suggested that the red pigment of Platypoecilus and Xiphophorus may belong in the melanin series. It is true that certain of the transformation products between tyrosine and melanin have a brown or red color (hallochrome), but the possibility that the red pigment may be a melanin seems to us to be improbable on the basis of the results of our investigation.

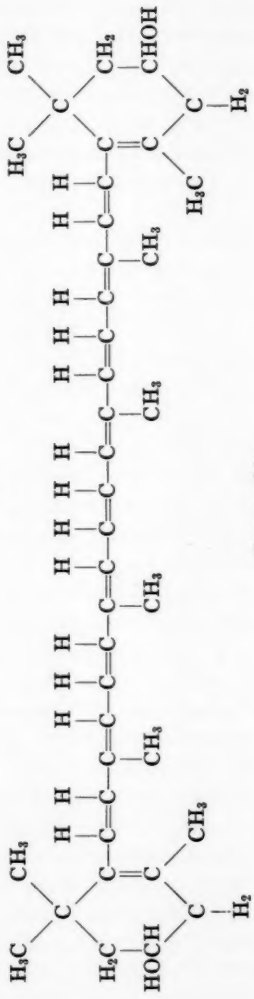
DISCUSSION

The three carotenoid pigments found in these fish are clearly similar xanthophylls. They were lutein (xanthophyll) $C_{40}H_{56}O_2$, zeaxanthin $C_{40}H_{56}O_2$, and violaxanthin $C_{40}H_{56}O_4$. The first two differ only by the position of a double bond. The structural formulae of these two is given below; that of violaxanthin is not known.

It was pointed out above that there were both red and orange cells in *Macropodus opercularis*, and this is also the case in *Colisa lalia*. These chromatophores seem to intergrade, although the intermediate cells are certainly less numerous than those of the two extremes. It may be that the two colors are due to the differing proportions of the two compounds lutein and violaxanthin and that one is a transformation product from the other. The intensity of color of these fish, and of other fish, seems to vary with age, metabolic conditions, and the breeding season or exposure to colored backgrounds (SUMNER 1940). These changes are not due merely to expansion or contraction of the cells but to the amount, intensity, and color of the pigment. A basis of such color changes may well be the existence of a series of transformable compounds such as those observed here. Many "improved" color strains of fish have been developed by fanciers, and it would be of interest to know of the existence of genetic color modifiers which may control these. So far, no genetic analyses have been made. It is perhaps not impossible, however, that fish may furnish material among the vertebrates which may show correlation between genetic constitution and the chemistry of pigments such as is known to exist in plants. (See reviews by LAWRENCE and PRICE 1940; SCOTT-MONCRIEFF 1937.)



Lutein



Zeaxanthin

TABLE 3

	YELLOW	RED OR ORANGE
Order Cyprinodontes		
Family Poeciliidae		
Platypoecilus maculatus (Platyfish)	Lutein Zeaxanthin	Erythropterin
Xiphophorus helleri (Swordtail)	Lutein Zeaxanthin	Erythropterin
Family Cyprinodontidae		
Subfamily Fundulinae		
Oryzias (Aplocheilus) latipes (Medaka)	Lutein	(No red pigment)
Order Labyrinthia		
Family Osphronemidae		
Macropodus opercularis (Paradise fish)	Lutein and violaxanthin	
Colisia lalia (Dwarf gourami)	Lutein and violaxanthin	
Colisia fasciata (Striped gourami)	Lutein and violaxanthin	
Betta splendens	Lutein	Erythropterin

As a matter of interest in relation to the problem of gene action it should be pointed out that in fish, as in many other forms, there is no "all or nothing" reaction. Most light colored fish have a few melanophores. There may possibly be a complete absence in a few true albino types as in Macropodus and in Xiphophorus (KOSSWIG 1935). Even the albinos, however, have xanthophores reduced in size and probably in number as compared with the yellow types. The gold types of Platypoecilus and Xiphophorus have a few xantho-erythrophores. We have no evidence of the quantitative action of a dominant gene causing incomplete dominance.

The pigment identifications reveal an expected similarity in the closely related types, Platypoecilus and Xiphophorus, which both possess the same three pigments—lutein, zeaxanthin, and erythropterin. Three of the four species of the family Osphronemidae—*Macropodus opercularis*, *Colisa lalia*, and *Colisa fasciata*—carry the pigment lutein and violaxanthin. The fourth species, *Betta splendens*, which is morphologically somewhat removed from the other three species, has a different assortment of pigments, lutein and erythropterin. Oryzias has only lutein. Thus there appears, ex-

cept possibly in the case of *Betta splendens*, a fair correlation with expectation of similarities based on taxonomic position. (See table 3.)

SUMMARY

Chemical identification was made of pigments present in the following fish: *Platypoecilus maculatus*, *Xiphophorus helleri*, *Oryzias latipes*, *Macropodus opercularis*, *Colisa lalia*, *Colisa fasciata*, and *Betta splendens*.

The pigments (other than melanin) appear to be the carotenoids, lutein (xanthophyll), zeaxanthin, violaxanthin, and the pterin, erythropterin. The carotenoids probably exist in the tissues in the form of esters.

The distribution of these pigments in relation to various mendelian color varieties of Platypoecilus, Xiphophorus, Oryzias, Macropodus, and Betta is outlined.

Platypoecilus and Xiphophorus varieties carrying the gene controlling the red color possess a peculiar cell, the xantho-erythrophore. This appears to be a xanthophore in which erythropterin is laid down in addition to the lutein and the zeaxanthin always present and is therefore the result of the specific action of this gene.

The relation of the distribution of pigments to the taxonomic position of the species is discussed. In the main, closely related types have the same pigments.

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MECHANISM OF PIGMENT FORMATION IN BOMBYX AND DROSOPHILA

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I. INTRODUCTION

MOST of the investigators engaged in studies of pigment formation in various insects have come to the view that the mechanisms have a common feature in that pigmentation is due to the combined functions of certain diffusible substances called gene-hormones and the receptive substrates or chromogens (EPHRUSSI and CHEAVAIS 1937; KAWAGUCHI 1938, et al.). My recent studies of pigment formation in Bombyx and Drosophila, however, have led me to reconsider the problem. Although these studies are still in progress, the principal results so far obtained will be given here.

II. GENERAL CONSIDERATIONS

I have reported in a previous paper (KIKKAWA 1940) that the v^+ substance of Drosophila is present in quantity in the body fluid of the pupa of Bombyx in the normal and some mutant types, as well as in their eggs directly after oviposition. The investigation has been extended to the eggs of various color types with the aim of ascertaining the biological relation between pigmentation in Bombyx and in Drosophila. The results obtained are shown in table 1.

As a rule the hibernating eggs of *Bombyx mori* are yellowish white when newly laid, but with elapse of time, pigments are gradually deposited in the serosa, and in about a week they acquire the characteristic color (blackish-violet in the wild type (Plate 1, fig. C), light brown in the brown-2k type (Plate 1, fig. D), etc). The unfertilized eggs of any type and the eggs of the white-1 and pink types maintain the original yellowish-white color for many months (Plate 1, fig. A).

It is of interest to note that the quantity of the v^+ or the cn^+ substance in Bombyx eggs is inversely proportional to that of the pigments in the serosa. Thus, the v^+ and cn^+ substances are present in quantity in the eggs of wild and red types directly after oviposition, but the old eggs which show their characteristic colors are completely or nearly lacking in these substances. The eggs of pink type and the unfertilized eggs of the wild type, however, retain both substances for about one hundred or even more days. These facts show clearly that the substances similar to the v^+ and cn^+ substances of Drosophila are indispensable to pigment formation in Bombyx eggs. This relation holds true for pigment formation in eyes

TABLE 1

Relations between the pigment formation of Bombyx mori and that of Drosophila melanogaster.

DONOR (Bomby x)						EYE COLOR OF HOST (Drosophila)	
Name	Symbol	Genetic formula	Days after oviposition	Egg color	Eye color of mother moth	Vermilion	Cinnabar
						Effect of donor extract on eye color of host	
Black or Wild	+	*WPR*	1–2	Yellowish-white	Black	+*	+
Do. (Fertilized)	+	*WPR*	6-over 100	Blackish-violet	Black	−(±)	−(±)
Do. (Unfertilized)	+	*WPR*	1–over 100	Yellowish-white	Black	+	+
White-1	*w*	*wPR*	1–2	Yellowish-white	White	+	−
Do.	*w*	*wPR*	6–over 100	Yellowish-white	White	+	−
Pink	*p*	*WpR*	1–2	Yellowish-white	Pink	+	+
Do.	*p*	*WpR*	6–over 100	Yellowish-white	Pink	+	+
Red	*r*	*WPr*	1–2	Yellowish-white	Red	+	+
Do.	*r*	*WPr*	6–over 100	Red	Red	−(±)	−(±)
Brown-2k	*bw2*	—	1–2	Yellowish-white	Black	−(±)	−(±)
Do.	*bw2*	—	6–over 100	Light brown	Black	−	−

Feeding method: See section IIIa.

* Plus sign under column headed Vermilion indicates donor contributed v^+ substance while minus sign indicates absence of this substance, etc. Likewise a plus sign under Cinnabar indicates the presence of cn^+ substance.

and other organs in Bombyx though the mechanism is apparently somewhat more complicated (unpublished).

As shown in table 1, brown-2k of Bombyx corresponds to vermilion of Drosophila which lacks both the v^+ and cn^+ substances, and white-1 of Bombyx corresponds to cinnabar of Drosophila which is deficient in the cn^+ substance. These two mutants of Bombyx are well known to geneticists and to breeders of silkworm, especially since they show peculiar maternal

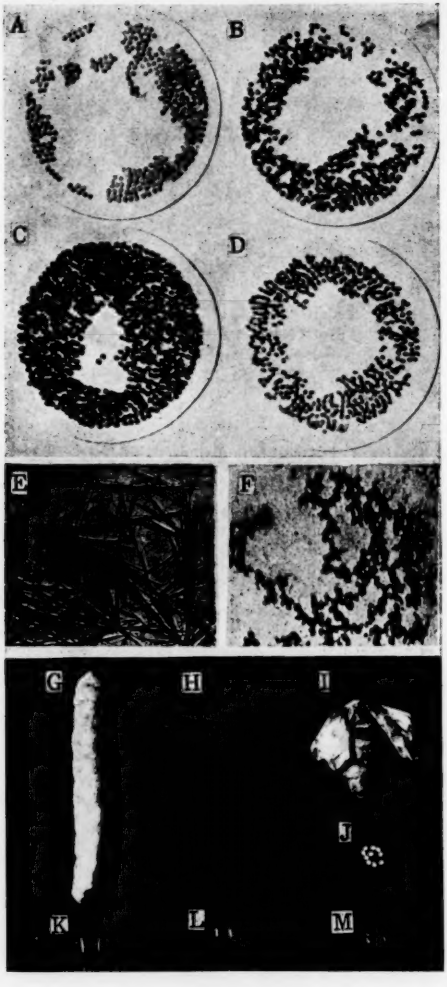
inheritance (TOYAMA 1912, 1913; UDA 1923, 1932; TANAKA 1924; KIKKAWA 1937; KAWAGUCHI and KIN 1938; UMEYA 1938; SUZUKI 1939; et al.). Strictly speaking, however, the genetical behavior of these two mutants is rather complicated, as will be shown in a separate paper. At any rate, one may notice from the above results that the mechanism of pigment formation in Bombyx is quite similar to that in Drosophila, Ephestia, and other insects thus far investigated (BEADLE, ANDERSON, and MAXWELL 1938; PLAGGE and BECKER 1938; et al.).

Biochemical studies of these substances involved in pigment formation in Bombyx and Drosophila have also been made. Pioneer work in this field has shown that the derivatives of tryptophane play an important part in this phenomenon (KHOUVINE, EPHRUSSI, and CHEVAIS 1938; TATUM 1939; TATUM and BEADLE 1940; BUTENANDT, WEIDEL and BECKER 1940a, b). Especially noteworthy are the results of BUTENANDT and others. They have shown that α-oxytryptophane and kynurenine are to be regarded as representing the v^+ substance in Drosophila and the A substance in Ephestia. But it has not yet been demonstrated that the wild types of these insects contain such tryptophane derivatives as kynurenine in their bodies. Furthermore, no substance corresponding to the cn^+ substance has been found.

Kynurenine, discovered by PROF. Y. KOTAKE of the OSAKA IMPERIAL UNIVERSITY, can be detected and estimated very accurately by the so-called Otani-Honda method (see section III b). Employing this method with various kinds of Bombyx eggs and Drosophila pupae, I have been able to detect the presence of kynurenine in the white-1 eggs of Bombyx and in the cinnabar pupae of Drosophila. Preliminary quantitative experiments have given the following results (Plate 1, fig. G–M): In Bombyx; 0.9–1.1γ per one egg. Since one female moth produces about 500 eggs, the amount of kynurenine in one batch should be 0.45–0.55 mg. This means that one gram of freshly laid eggs contains 1.6–2.0 mg of kynurenine (in one gram there are about 1800 eggs). In Drosophila; 0.1–0.2γ per pupa, and 0.08–0.17 mg per one gram of fresh pupae (in one gram there are about 830 pupae).

Thus, when a constant weight of material is taken into consideration, the amount of kynurenine in the cinnabar pupae of Drosophila is much less than in the white-1 eggs of Bombyx. This difference may be partly due to the fact that in Drosophila pupae the substance is being formed, while in Bombyx eggs the necessary amount has already been formed and preserved. Then the total amount of kynurenine produced by *cn* pupae of Drosophila would be greater than that estimated above.

Knowing that kynurenine is contained in quantity in the white-1 eggs of Bombyx, an attempt was made to obtain it in a pure state. I have suc-



EXPLANATION OF PLATE 1

FIGURES A–D. Bombyx eggs. ×0.7.—A. White-1 eggs (Eggs directly after oviposition of any type, unfertilized eggs, and pink type eggs give similar yellowish-white colors).—B. Variegated eggs (White-1 eggs modified by implanting an ovary having the W_1 gene).—C. Black or wild type eggs.—D. Brown-2k eggs.

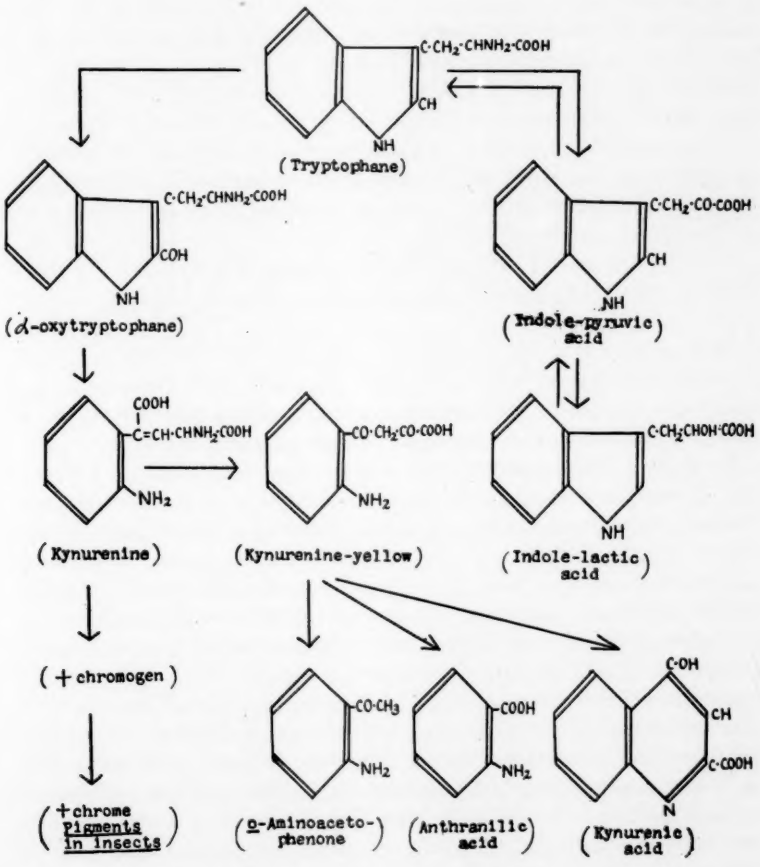
FIGURE E.—Crystals of kynurenine-sulphate obtained from the White-1 eggs of Bombyx. ca. ×100.

FIGURE F.—+ chromogen granules (?) obtained from the wild type eggs of Bombyx. ca. ×800.

FIGURES G–M.—Comparisons of the larva, pupa, imago, and eggs of *Bombyx mori* with those of *Drosophila melanogaster*. ×0.7.—G–J. Bombyx.—G. Larva of the fifth instar.—H. Pupa.—I. Imago (Female).—J. Egg.—K–M. Drosophila.—K. Larva of the third instar.—L. Pupa.—M. Imago.

ceeded in getting the substance in the form of kynurenine-sulphate (Plate 1, fig. E) (see section III e). A solution of this crystal showed the specific reaction of kynurenine by the Otani-Honda method and gave a strong effect on the *v bw* larvae of Drosophila.

Derivatives of tryptophane.



It was further noted that kynurenine had been detected neither in the fresh eggs of the wild and other mutant types of Bombyx nor in the wild type pupae of Drosophila. Since it is already clear that these materials contain both the v^+ and cn^+ substances in quantity (table 1), it is evident

that kynurenine has been converted into a different substance which has both the v^+ and cn^+ effects, and further that the cn^+ gene of Drosophila and the W_1 gene of Bombyx are indispensable to this conversion. What, then is this substance? According to PROF. KOTAKE and his coworkers (KOTAKE et al. 1938, KOTAKE 1940), tryptophane is converted or derived from other substances by the following processes.

Of these substances, kynurenine-yellow, *o*-aminoacetophenone, anthranilic acid, and kynurenic acid are of special interest, for they are close derivatives of kynurenine, and they all satisfy the well known condition that the cn^+ substance is derived from the v^+ substance (EPHRUSSI and CHEVAIS 1937; TATUM 1939; et al.). There is, however, *a priori* evidence that these substances are not regarded as the cn^+ substance, inasmuch as they are soluble in ether except the last one—in acid ether at least. In fact BUTENANDT et al. (1940b) showed that kynurenic acid was ineffective on both the *v* and *cn* larvae. I have also obtained a negative result using anthranilic acid on them.

Thus we had to look elsewhere for the substance in question. Ehrlich's diazo reaction finally gave the clue (see section III c, d). The idea was suggested to me by PROF. KOTAKE'S (1940) paper, in which he and his coworkers showed that WEISS'S urochromogen and SACHS'S color substance were probably derived from tryptophane, especially from kynurenine, and that these substances were regarded as representing the foundations of Ehrlich's diazo reaction in the urine (WEISS 1922; SACHS 1936).

Employing this technique with Bombyx eggs and Drosophila pupae, the following results were obtained. The water or alcoholic extracts of Bombyx eggs directly after oviposition showed the diazo reaction very clearly, but the extracts from eggs of white-1 and brown-2k, both lacking the cn^+ substance, gave no, or only a weak, reaction. The reaction was also positive to the extracts of old eggs of the pink type which do not deposit any brown pigment in the serosa. A similar result was obtained for old but unfertilized eggs of the wild type, while the reaction diminished gradually with time when the test was made on the fertilized eggs of the wild type. This relation is quite in accordance with the result obtained in my previous paper (1940) in which I showed that the v^+ substance decreased gradually with time and largely disappeared within a week. When the intensity of diazo reaction in a certain condition (see section III c) was taken as the standard (100 and 0), the following quantitative relations were found in the eggs of various types (table 2).

Comparing the results in table 2 with those in table 1, we can see clearly that the substance showing Ehrlich's diazo reaction is identical with the cn^+ substance. But, since it has the v^+ activity as well, henceforth we shall call the substance + chromogen.

TABLE 2

Diazo values of + chromogen in various Bombyx eggs.

NAME	SYMBOL	DAYS AFTER OVIPOSITION	EGG COLOR	DIAZO VALUE
Black or Wild*	+	1	Yellowish-white	100
Do.	+	2	Reddish-brown	40.1
Do.	+	3	Blackish-violet	18.1
Do.	+	4	Blackish-violet	15.1
Do.	+	5	Blackish-violet	16.7
Black† (Fertilized)	+	40	Blackish-violet	17.6
Black (Unfertilized)	+	40	Yellowish-white	100
White-1	*w*	ca. 200	Yellowish-white	0.0
Pink	*p*	ca. 200	Yellowish-white	96.5
Red	*r*	ca. 200	Red	21.4?‡
Brown-2k	*bw2*	ca. 200	Light brown	24.3

Diazo values were obtained by the method described in section III c.

* Eggs were kept at 26.5°C.

† A strain different from that in the above experiment.

‡ Since the original solution usually showed a darker color than the standard solution, this value is doubtful.

Water or alcoholic extracts of *v* or *cn* pupae of *D. melanogaster* do not show this reaction, while those of the wild type, brown, white (the reaction is weak) or Bar pupae do. Similarly, the *v* mutants of *D. ananassae*, *D. montium* and *D. virilis* give negative results, while the wild types of all these species give positive results.

Ehrlich's diazo reaction, as well as Pauly's, is not specific for + chromogen and analogous substances, because imidazol derivatives, like histidine, and *p*-oxyphenyl derivatives, like tyrosine, show similar reactions. But the experimental results shown above testify that the positive reaction can hardly be attributed to the presence of histidine or tyrosine. Furthermore, Ehrlich's diazo reaction for Bombyx eggs is rather specific—the solution does not lose the characteristic reddish tone even if it is greatly diluted with water; but the solution becomes yellowish in case of histidine or tyrosine.

Direct evidence that kynurenine had been converted into the substance showing Ehrlich's diazo reaction was obtained by the following experiments. When the color of white-1 eggs of Bombyx is modified by implanting an ovary having the W_1 gene (KIKKAWA 1937, and Plate 1, fig. B in this paper), the water extract of these fresh eggs shows both the Otani-Honda reaction for kynurenine and Ehrlich's diazo reaction for + chro-

mogen. However, with deposition of brownish pigments in the serosa, Ehrlich's diazo reaction disappears and only the kynurenine reaction remains. The amount of kynurenine in the grayish eggs, however, is apparently less than in the control white-1 eggs—for example, 0.61 mg per 1000 eggs in the former and 0.99 mg in the latter. This result indicates clearly that about 38 percent of the kynurenine in the white-1 eggs had been converted into the + chromogen.

Similarly, when the *v bw* larvae of Drosophila are raised on the medium containing kynurenine (about 2 mg per 5 cc of medium), the water extract of the pupae or the body fluid shows Ehrlich's diazo reaction. These facts indicate that in order to form pigments, kynurenine is converted into + chromogen in the body of the insect.

In connection with this experiment, I have determined the minimum amount of kynurenine capable of giving an effect on the *v bw* larvae. In table 3 and figure 1 are summarized the results obtained (the eye color value was calculated by the method described in section III a).

TABLE 3

Effects of concentration of kynurenine on eye color of v bw flies.

AMOUNT OF KYNURENINE PER 5 CC MEDIUM	EYE COLOR VALUE	NUMBER OF FLIES
0.00 mg.	0.0	46
0.03	0.0	20
0.08	0.1	20
0.14	0.5	12
0.28	1.4	28
0.55	2.1	22
1.40	2.5	18
2.80	3.0	20

Feeding method: See section III a.

Since the minimum amount of kynurenine which can be detected by the Otani-Honda method (hot method) is regarded to be about 0.125 mg per 5 cc solution, it may be safely concluded that the biological method shown above is rather accurate and convenient for the detection of kynurenine. However, it should be noted that the color values are in direct proportion to the amount of kynurenine contained in the medium only within certain limits.

Various attempts were made to isolate the + chromogen. Although the substance may be detected in the fraction treated similarly with kynurenine, I have not yet succeeded in obtaining it in crystal form. It has been obtained only in a form of very small granules of the diameter about 0.8μ, which are nearly colorless (Plate 1, fig. F). Unfortunately, the purity of

this substance is doubtful, since the solution used for the test might have contained other organic substances which resemble the + chromogen, the so-called Kotake's kynurenine-bodies. Yet the substance has properties in common with those of WEISS's (1922) urochromogen and SACHS's (1936)

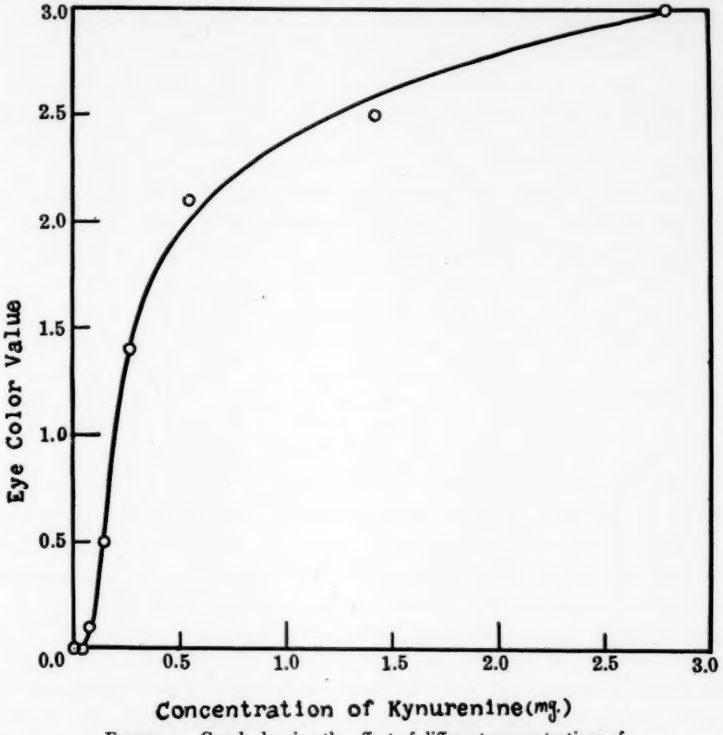


FIGURE 1.—Graph showing the effect of different concentrations of kynurenine on eye color of *v bw* flies.

color substance. For the sake of comparison, the properties of kynurenine and + chromogen are shown in table 4.

As far as my tests go, the + chromogen seems to have no neutral sulphuric molecule detected by the PbS method. In this respect, the + chromogen seems to be distinguished from WEISS's urochromogen. In fact, the attempt to modify the eye color of *v* and *cn* larvae by using tuberculous urine showing Ehrlich's diazo reaction failed, though this result must not yet be considered conclusive. Besides, the characteristic that the + chromogen is insoluble in acid ether shows a distinction between the chromogen in question and SACHS's color substance.

TABLE 4

Comparisons of the properties of kynurenine with those of + chromogen.

	KYNURENINE	+ CHROMOGEN
Molecular formula	$C_{11}H_{12}N_2O_4$	Unknown*
Molecular weight	236.1	ca. 400–600†
Crystal form	Plate-like; Needle-like as sulphate	Granules ?
(Color reactions)		
Otani-Honda reaction	+	−
Ehrlich diazo reaction	−	+
Weiss $KMnO_4$ reaction	+	+
Xanthoprotein reaction	+	+
Millon reaction	−	−
Biuret reaction	−	−
Ninhydrine reaction	+	+
Molisch reaction	−	−
Adamkiewicz reaction	−	−
Neubauer-Rohde reaction	−	−
PbS reaction	−	−
Precipitated with	Phosphotungstic acid, Mercury sulphate, Basic lead acetate, etc.	The same
Reducing power‡	Weak	Strong

* SACHS' kynurenine derivative: $C_{19}H_{20}N_2O_7$.

† After TATUM and BEADLE (1938) and BECKER (1939).

‡ Reducing power was measured by the formation of Prussian-blue in the mixture of dilute solutions of potassium ferricyanide and ferric chloride (Ferri-ferri reaction).

At any rate, the + chromogen seems to be characterized by the following chemical properties: It is detected by Ehrlich's diazo reaction; it is soluble in water and alcohol, but insoluble in ether, benzene and chloroform; it has a strong reducing power; it has at least a benzene nucleus; it is precipitated with phosphotungstic acid or with mercury sulphate; it sets free NH_3 and a jasmine-like aroma in hot basic solution; it is stable in acid solution, but rather unstable in a basic one. Some of these properties have already been recognized by previous investigators. Precise chemical investigations on the + chromogen are being carried out by DR. S. OTANI of the OSAKA IMPERIAL UNIVERSITY under the direction of PROF. Y. KOTAKE.

As suggested before, the + chromogen seems to be converted into the + chrome—that is, pigments found in the eye, serosa, ganglion, Malpighian tubules, gonad, and even in a part of skin cells, by the activities of various enzymes. The so-called + chrome is probably identical with

BECKER's skotommin found in Ephestia (BECKER 1939) or with COCHRANE's brown and red pigments found in Drosophila (COCHRANE 1937). So far we have no precise knowledge of the process of conversion from + chromogen into + chrome, or of the chemical nature of + chrome. But it has been confirmed that the + chrome is insoluble in water and alcohol but soluble in dilute alkaline solution, and further that it seems to be composed of large molecules and probably belongs to a kind of chromoproteins.

Based on the facts mentioned above, we can assume that the following conversions go on in the pigment formation in those insects: Kynurenine → + chromogen→ + chrome.

Next, let us consider the processes which take place prior to the formation of kynurenine. Unfortunately, this point has not been clarified thoroughly for Bombyx, although the following facts have been ascertained for some other insects. According to BUTENANDT et al. (1940b), α-oxytryptophane is capable of taking the role of the v^+ substance in Drosophila and of the A substance in Ephestia, though it is weaker in effect than kynurenine. These authors have assumed, accordingly, that the *v* or *a* mutants are probably deficient in the enzyme which oxidizes the α-position in the pyrrole nucleus of tryptophane. This may be true, but there are still other possibilities. For example, there is the possibility that the enzyme activity which converts α-oxytryptophane into kynurenine, KOTAKE and MASAYAMA's (1938) tryptophane-pyrrolase, is weaker in those animals than that in the wild type animals. This point remains to be examined thoroughly.

In connection with the above problem, it is to be noticed here that tryptophane and some other indole derivatives are entirely ineffective on both the *v* and *cn* larvae of Drosophila. Many trials by the writer employing *l*-tryptophane, *d*-tryptophane, indole-lactic acid, indole-propionic acid and indole-acetic acid (heteroauxin) have yielded only negative results (unpublished). The positive result reported by KHOUVINE, EPHRUSSI, and CHEVAIS (1938) is apparently due to the intervention of some microorganisms growing in the media, for it is well known that some microorganisms like *Bacterium subtilis* and *B. mesentericus* can produce kynurenine or its analogous substances from tryptophane. This fact has been pointed out by TATUM (1939).

The most important and interesting problem awaiting solution is the origin of the tryptophane. As stated before, free tryptophane is probably required as the primary substance in pigment formation. But as far as my examinations go, free tryptophane or indole derivatives cannot or hardly can be recognized in the body fluid of Bombyx deproteinized with ethanol at any stage in development, in spite of the fact that the proteins precipitated clearly show the tryptophane reaction. Similar results have been obtained for the alcoholic extract of various types of Drosophila

(*v*, *cn* and +). Such observations may be partially accounted for by the assumption that the free tryptophane which is produced in the body fluid or in various cells is converted immediately into other substances like kynurenine, + chromogen or some other unknown derivatives. This assumption, however, is incapable of explaining the problem of how the large quantity of free tryptophane is produced in the pupal stage. Several investigators have shown that the color substance concerned is entirely or nearly absent in the larval stage of the insects, but is found in quantity in the pupal stage (hence, the necessity of the free tryptophane), notwithstanding that the animals take no nourishment during this stage (HARNLY and EPHRUSSI 1937; KIKKAWA 1940). To solve this problem, we must assume that the free tryptophane is produced by either the decomposition of proteins or the synthesis from other substances. These assumptions are both possible. In connection with this problem, it is of interest to recall the assertion of JEZEWSKA (1926) that the total amount of tryptophane increases in the early stages of pupal life of *Musca vomitoria*.

Thus, in the present state of our knowledge bearing on this problem, we may assume the following system operating in pigment formation: Tryptophane→ α-oxytryptophane→ kynurenine→ + chromogen→ + chrome (pigment). No doubt, with the progress of our knowledge in this field, there will be found many more intermediate links to be added to the chain of the whole system.

It is hardly to be doubted that enzymes play an important role in the above conversions. If we call the enzymes concerned with these changes enzymes I, II, III, and IV, respectively, the presence or absence of these enzymes is probably controlled by genes like v^+ and cn^+ in Drosophila and W_1 and P in Bombyx, which act independently of one another. This relation is indicated schematically in table 5.

Looking at this table, one may notice that the system shown above is somewhat different from that proposed by previous investigators. Namely, the substances called gene-hormones by them are regarded here as representing the substrates of pigments or chromogen-substances, and the substrates or chromogens of those authors are here represented by the enzymes controlled by genes. A similar assumption was previously made by SCHUURMAN (1937), BUTENANDT et al. (1940a), and also by KOTAKE (1940).

The enzymes concerned, especially enzyme IV, seem to belong to the category of intracellular enzymes (lyo, endo, or desmo) because mosaicism in the pigmentation of eyes and eggs frequently occurs (VAN ATTA 1932 in Drosophila, TAZIMA 1939 in Bombyx). In connection with this problem it is of interest to indicate the following facts. When the *cn* larvae of Drosophila are raised on a medium containing *l*-tryptophane, the water extract

of the pupae shows a stronger reaction of kynurenine than that of the control (*d*-tryptophane is ineffective!). This finding suggests strongly that the *cn* type has the enzymes I and II which correspond to KOTAKE and MASAYAMA'S tryptophane-pyrrolase, which was obtained from the glycerine extract of the liver of the rabbit.

Similarly, when the wild type larvae of Drosophila are raised on the *l*-tryptophane medium as described before, the water or alcoholic extract

TABLE 5

Schematic system in relation to the pigment formation in the insects.

NAME OF INSECT	NAME OF MUTANT	ORDER OF CONVERSION: Tryptophane→α-oxytryptophane→Kynurenine→+chromogen→+chrome (Pigment)			
		↑ Enzyme I	↑ Enzyme II	↑ Enzyme III	↑ Enzyme IV†
Bombyx	Black (Wild)	+	+	+	+
	White-1	+	+	−	+
	Pink	+	+	+	−
	Brown-2k*	±	±	±	±
Droso-phila	Vermilion	−	±?	+	+
	Cinnabar	+	+	−	+
	White	±	+	±	−
	Wild	+	+	+	+
Ephestia	Red	−	±?	+	+
	Black (Wild)	+	+	+	+

* Ovarian cells only. The body fluid in the pupa of this mutant has + chromogen in quantity, and the eye of mother moth is black.

† The enzyme is not of one kind; it is peculiar to each mutant.

of the pupae shows a stronger reaction of + chromogen than that of the control. In this case, the kynurenine reaction is positive, too. These facts suggest that the wild type has the enzymes I, II, and III in the body.

The system shown above may be applied not only to such insects as Bombyx, Drosophila, Ephestia, Habrobracon, and Tenebrio, but also to various insects and other arthropods. In fact, I have found that the + chromogen is present in the pupae of Ephestia, Antheraea, and in about ten insect species of the wild type.

Before concluding this section I should like to point out the following phenomenon. As shown by KÜHN (1937) and others, the *a* animal of Ephestia is less vigorous than the wild type (*A*), although no such difference in vitality may exist between the *v*, *cn* mutant and wild types of Drosophila. But a similar low vitality is recognized in the white-1 and other eye and egg color mutants of Bombyx. Possibly the abnormalities in the

tryptophane metabolism in these mutants has a deleterious influence on intracellular respiration. We should recall that such color substances as cytochrome, hallachrome, and lyochrome play an important role for the redox-system of animals.

III. EXPERIMENTAL METHODS

In this section are given the experimental methods used in the present study. No doubt, the methods still require much improvement in future studies.

(*a*) *Feeding method for Drosophila*

In general (KIKKAWA 1940), into a vial (diameter 2 cm; height 7 cm; volume ca. 22 cc) is pipetted 5 cc culture medium made up of the following mixture: 4 cc of standard glucose-agar solution (ratios: glucose 2 g, agar 1 g, water 30 cc); 0.6 cc of phosphate-buffer solution adjusted to a pH of 5.91; 0.4 cc of the solution to be tested (body fluid, egg-extract, etc.). To the medium is added a small amount of brewer's dry yeast with a drop of water and a piece of filter paper. Fifteen test larvae (ca. 72 hours after egg laying at 25°C) are placed in each vial. All the experiments are carried out at 25°C. After the emergence of flies, they are divided into five classes according to the grade of eye color; 0 (no response), 0.5, 1.0, 2.0, 3.0 (identical with *bw*).

Referring to the eye colors of *v bw* (*cn bw*) and of *bw*, the standard may be constructed by combining in proper portion the phenol-red solution with the buffer solutions adjusted to pH of 7.0, 7.4, 7.8, 8.0, and 8.3, respectively. In special cases, some more complicated methods are necessary.

(*b*) *Otani-Honda method for kynurenine-detection and the quantitative method* (*Modified by the author*):

Reagent I. 15 percent HCl containing 2 percent p-dimethylaminobenzaldehyde.

Reagent II. 3 percent hydrogen peroxide.

For Bombyx

Three hundred eggs are placed in hot water at 90°C for five minutes, and after being dried on filter paper, crushed with 1.5 cc of 2.5 percent metaphosphoric acid. The colloidal solution is heated on a water-bath at 70°C for three minutes, then centrifuged, and 1 cc of the filtrate is used. To the solution to be tested is added 0.2 cc of reagent I (the solution becomes yellowish color at this time if kynurenine and the analogous substances are present) and 0.2 cc of reagent II.

The mixed solution is then heated for 20 minutes on a water-bath at 70°C. The solution shows a beautiful violet color if kynurenine, anthranilic

acid, or *o*-aminoacetophenone is present. After cooling the solution is shaken with ether in order to remove excess reagents and other unpurified substances. After removal of ether, the solution is shaken several times with butanol saturated with water. The violet color due to kynurenine is left in the water solution, while that due to anthranilic acid or to *o*-aminoacetophenone is transsolved into the butanol. After removal of the butanol, 5 cc of water is added to the solution and its color compared with that of the standard solution (0.43 mg of kynurenine per 1 cc), which was treated with the same procedure, by Dubosque's colorimetric analysis (Purflich's photometric analysis is more adequate). OTANI and HONDA (1938) state that the keenness of this method is increased by leaving the solution at room temperature for 14 to 20 hours instead of heating it.

For Drosophila

On account of the small quantity of kynurenine per each *cn* pupa, detection and estimation are both difficult. Three hundred *cn* pupae, 72 hours after pupation at 25°C, are used and prepared with a procedure similar to that described before except that 2 cc of 2.5 percent metaphosphoric acid is used in this case. Then to the solution to be tested (1 cc) is added 0.3 cc of reagent I and 0.3 cc of reagent II. The subsequent procedures are the same as those described above. The standard solution used here contains only 0.03 mg of kynurenine per cc. For the control the *v* and + pupae of the same age are used, both being negative to the reaction.

(c) Ehrlich's or Pauly's diazo reaction method for + chromogen and the quantitative method

Reagent I. 1.75 percent HCl containing 0.5 percent sulphanilic acid.
Reagent II. 0.5 percent sodium nitrite.
Reagent III. 35 percent NH_4OH or saturated Na_2CO_3 solution.
Mix the reagents I and II in the ratio of 40 to 1.

For Bombyx

Three hundred wild type eggs directly after oviposition are placed in hot water at 90°C for five minutes, dried on filter paper, and then crushed with 2 cc of 80 percent ethanol (sometimes 2 cc of distilled water was used instead of ethanol). The colloidal solution is heated on a water-bath at 70°C for three minutes, centrifuged, and 1 cc of the filtrate is used. To this solution is added 1 cc of the mixture of reagents I and II and 0.3 cc of saturated Na_2CO_3 solution (for a simple qualitative method a few drops of 35 percent NH_4OH solution are used).

The solution shows a beautiful scarlet color if the + chromogen is present in quantity. After visual observation, 3 cc of distilled water is

added to the solution. The color of the solution after 20 minutes is regarded as representing the value 100 (the scarlet color of the solution fades gradually, and the solution becomes brownish yellow with a slight turbidity). On the other hand the color of the solution where 300 white-1 eggs were used is taken as representing the value 0.0 (the color is of light yellow with a slight turbidity).

TABLE 6

The relation between the diazo values and the numbers of Bombyx eggs directly after oviposition.

NUMBERS OF EGGS DIRECTLY AFTER OVIPOSITION	DIAZO VALUE
Control (300 w_1 eggs)	0.0
50	21.1
100	38.9
200	64.1
300	100.0

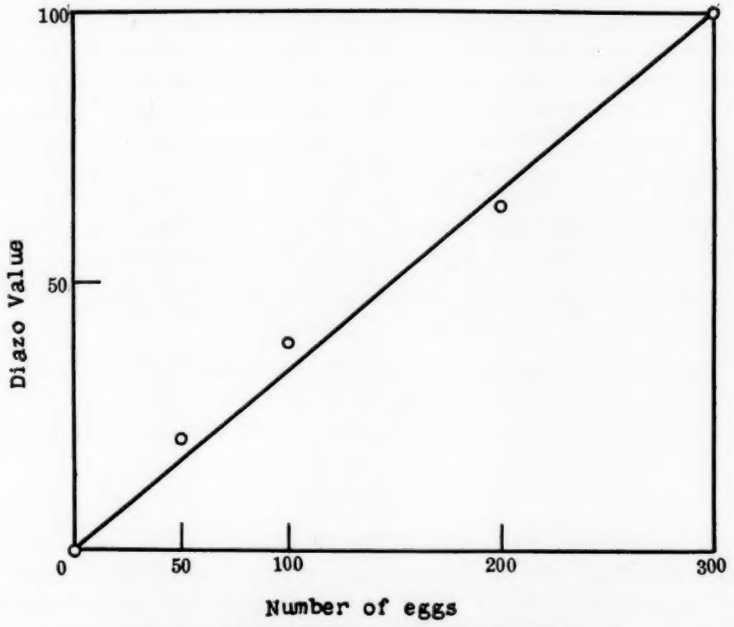


FIGURE 2.—Figure showing the relation between the diazo values and the numbers of Bombyx eggs.

Taking these two colors as standard values for diazo reaction, the colors

in cases where other mutant eggs (300 eggs) are used is estimated by Dubosque's colorimetric analysis (table 2).

The validity of this quantitative method has been substantiated by the fact that the diazo values calculated by the above method are directly proportional to the numbers of eggs used (table 6 and fig. 2).

For Drosophila

Three hundred pupae about 72 hours after pupation at 25°C (*v*, *cn*, *w*, *bw*, and + pupae) are placed in hot water at 90°C for three minutes and, after being dried on filter paper, are crushed with 2 cc of 80 percent ethanol. The subsequent procedure is quite similar to that for Bombyx eggs. Since the diazo value for the + pupae of Drosophila is too small to be estimated, no quantitative study has been performed. Yet the response of the *v* and of the *cn* pupae to this reaction was found to be negative. The reaction for the *w* pupae was very weak.

(*d*) *A simple method for Ehrlich's or Pauly's diazo reaction*

In order to test a small quantity of human urine, HAMADA (1936) has proposed a simple method in which a filter paper moistened with the mixture of reagents I and II is used. I have applied this simple method to Bombyx eggs and Drosophila pupae with success. On a filter paper several Bombyx eggs or Drosophila pupae (sometimes only the body fluid was tested by the capillary method) are crushed in a drop of the mixture of reagents I and II with a glass rod. Then a drop of 15 percent Na_2CO_3 or 15 percent NH_4OH solution is added (a concentrated alkali-solution is to be avoided here). The filter paper shows a scarlet color if the + chromogen is present. Thus this method is similar to the experimental strip method used frequently in the studies of Tenebrio and some other insects. If the standard colors are determined properly by combining the phenol-red solution with buffer solutions of various pH values as stated in (a) of this section, we may use this as a simple quantitative method.

(*e*) *Purification of kynurenine and + chromogen from Bombyx eggs*

(1) About 20 grams of eggs (ca. 36000 eggs) are used in each experiment. The eggs are placed in hot water at 90°C for five minutes, dried on filter paper, crushed in 150 cc of 80 percent ethanol, heated on a water-bath at 70°C for 20 minutes, and kept at room temperature for 24 hours. Then the proteins and other insoluble substances are removed by centrifuging. The alcoholic part of the filtrate is evaporated on a water-bath at 60°C under reduced pressure. The filtrate is then shaken with ether several times in order to remove the ether-soluble substances. Then the filtrate is acidified

with 25 percent H_2SO_4 till the solution contains 5 percent H_2SO_4. This solution is shaken with ether once more, and to this acid solution are added twofold volumes of 5 percent H_2SO_4 solution containing 20 percent phosphotungstic acid. After 48 hours the precipitate is collected by centrifuging and then washed several times with 5 percent H_2SO_4 solution containing 2 percent phosphotungstic acid. The precipitate is then suspended in a small quantity of 5 percent H_2SO_4 solution and shaken with an equal volume of the mixture of amyl alcohol (1.0) and ether (1.25), in order to remove the phosphotungstic acid. The treatment is repeated three times.

The acid solution with kynurenine is neutralized with $Ba(OH)_2$ or $BaCO_3$ till it shows a slight acidity for congo-red paper (pH about 4.0). The solution is then evaporated on a water-bath at 60°C under reduced pressure till it becomes a small quantity, and is kept at the room temperature for 24 hours. Crude crystals of kynurenine-sulphate with mixture of other organic and inorganic salts are obtained. The crystals are dissolved in hot 60 percent ethanol to remove insoluble salts. By repeating this procedure, kynurenine-sulphate may be obtained in nearly pure state.

However, I have been unable to obtain the crystals of + chromogen as sulphate. The final solution was neutralized with $BaCO_3$ till the pH value became 6.0 and was shaken several times with butanol which was saturated with water, as done by TATUM and BEADLE (1938). But even these procedures have not enabled me to obtain crystals of the + chromogen.

(2) Another purification technique is used frequently by PROF. KOTAKE and his coworkers. In this method, Hopkins-Cole's reagent (5 percent H_2SO_4 solution containing 10 percent $HgSO_4$) is used instead of phosphotungstic acid. The precipitate thus obtained is suspended in a small quantity of water and treated with H_2S. The filtrate is treated with the method described above. I tried this method several times, and obtained results similar to that described in (1).

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V. SUMMARY

The mechanism of pigment formation in the eggs and various organs of Bombyx is quite similar to that known in Drosophila, Ephestia, and other insects. Biochemical studies have been carried out on Bombyx and Drosophila.

The presence of kynurenine and its derivatives + chromogen and + chrome was ascertained. Kynurenine was detected by the Otani-Honda method from the white-1 eggs of Bombyx, and + chromogen from normal and various color mutant eggs by Ehrlich's diazo reaction method. These substances, especially kynurenine, were obtained in a pure state from Bombyx eggs; they all give strong effects on the *v* or *cn* larvae of Drosophila.

In Drosophila kynurenine was detected in the *cn* pupae, and + chromogen in the *w* (the reaction is weak), *bw*, and wild type pupae. The *v* mutant lacks both kynurenine and + chromogen.

These findings, in combination with those by BUTENANDT et al. (1940a, b) in Drosophila and Ephestia, enable us to assume the following system as to the pigment formation in the insects: Tryptophane→ α-oxytryptophane→ kynurenine→ + chromogen→ + chrome (pigment).

The mechanism of the conversions from tryptophane into + chrome is assumed to be due to the presence of enzymes. These enzyme functions seem to be controlled by the genes like v^+, cn^+ and w^+ in Drosophila, and those like W_1 and P in Bombyx. The system is schematically shown in table 5.

The experimental methods which were used in this paper are briefly described.

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GENETIC CHARACTERISTICS OF THE B CHROMOSOMES IN MAIZE[1]

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MAIZE normally has ten pairs of chromosomes, but supernumerary or extra chromosomes known as B-type chromosomes (RANDOLPH 1928b) are of frequent occurrence in various commercial varieties and genetical cultures. First reported by KUWADA (1915) in sweet corn nearly a quarter of a century ago, they have since been discovered, not only in sweet corn (LONGLEY 1927, FISK 1927, RANDOLPH 1928a, AVDULOW 1933, HUMPHREY 1935), but also in flint corn (RANDOLPH 1928a, KOZHUCHOW 1933), flour corn (LONGLEY 1927, 1938), dent corn (LONGLEY 1927, KOZHUCHOW 1933), and pop corn (KOZHUCHOW 1933). In a survey of maize varieties grown by North American Indians, LONGLEY (1938) noted the presence of B chromosomes in 14 of 33 varieties in each of which from two to 32 plants were examined. Among 440 plants of these varieties of Indian maize, there were 63 with one or more B chromosomes.

There is some evidence that the B chromosomes are more prevalent in certain types of maize than in others. In most commercial stocks of dent, pop, and flour corn they have been found very rarely. KIESSELBACH and PETERSEN (1925) failed to find any deviation from the typical number in 11 commercial varieties and eight inbred strains of dent corn grown in Nebraska, and in the writer's experience B chromosomes have never been seen in commercial varieties and inbred lines of dent corn commonly grown in the northeastern United States, although many have been examined over a period of years. However, LONGLEY (1927) found B chromosomes in a long-time inbred strain of Crosby dent corn, and KOZHUCHOW (1933) reported their presence in the commercial dent varieties Minnesota 23 and Sterling. Data obtained from counts of relatively small numbers of individuals suggest that the B chromosomes are somewhat more prevalent in flint corn than in dent corn, and it is quite probable that they actually are more prevalent in sweet corn, especially the varieties Black Mexican and Golden Bantam, than in other types of maize. Most individuals grown from commercial seed of the variety Black Mexican ordinarily have from one to several B chromosomes, and numbers as high as eight to 12 have been reported (RANDOLPH 1928a, AVDULOW 1933). HUMPHREY (1935) noted the

[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, UNITED STATES DEPARTMENT OF AGRICULTURE, and the NEW YORK STATE COLLEGE OF AGRICULTURE, Ithaca, New York.

presence of B chromosomes in each of 11 inbred lines of Black Mexican which were investigated. In the flint types that have been examined, only occasional individuals are found with B chromosomes, and ordinarily not more than from one to three are present in such exceptional plants.

The existence of an equilibrium distribution of the B chromosomes in different stocks was postulated recently by DARLINGTON and UPCOTT (1941), and it was suggested that genetic function could be inferred from such population studies, although genetic activity in individual plants might not be detected. This is an interesting suggestion, and it is to be regretted that the available data on the frequency of B chromosomes in different types of maize are too fragmentary to provide an adequate basis for such population analyses. A simpler approach to this general problem and one that is cytologically more feasible is through the analysis of hybrids between types of maize in which B chromosomes are rare, as for example, dent types, and types such as sweet corn in which they are more prevalent. Studies of this sort have been made and will be discussed in some detail later in this paper; but the fact is that the B chromosomes appear to have no greater deleterious effects when introduced into the germplasm of dent maize than they ordinarily have in sweet corn.

There has been much speculation about the origin and fundamental nature of the B chromosomes. The solution of the problem has been approached up to the present time chiefly from the cytological angle. It has been established that they are very different from the normal A chromosomes both in appearance and behavior in the life cycle. But the cytological observations have failed to produce even a satisfactory working hypothesis as to their origin.

APPEARANCE OF THE B CHROMOSOMES

In somatic metaphases the B chromosomes are readily distinguishable from the A chromosomes. They are shorter and stain more intensely in preparations fixed in Craf (RANDOLPH 1935) or other chrom-acetic-formalin mixtures. The B chromosomes are typically club-shaped due to the accumulation of heterochromatin at the distal end, and there is a marked tendency for them to be grouped at the periphery of the somatic metaphase plates, a characteristic also noted by AVDULOW (1933). In an earlier paper (RANDOLPH 1928a) it was stated that in root-tip preparations the B chromosome appeared to have a terminal fiber attachment; and so it does. But in the pachytene stage where the relative position of the centromere can be more accurately determined, it is apparent that a rudimentary second arm is present, at least in many preparations.

The occurrence of distinctly two-armed B-type chromosomes in maize has been described from somatic figures by DARLINGTON (1937), AVDULOW

(1933), and others in recent years. But in these cases the position of the centromere very probably has been misinterpreted. The typical B chromosome often exhibits in root-tip figures what appears to be a subterminal constriction, especially after fixation with fluids that shrink the chromosomes. This is not a true centric constriction but is actually the weakly chromatic region between the proximal knob adjoining the centromere and the distal heterochromatic portion of the chromosome. This interpretation is quite obvious if one is familiar with the pachytene structure of the B chromosome and with the transformation accompanying its shortening during the late prophase and early metaphase of the first microspore division. In these stages the distinction between euchromatin and heterochromatin is clearly apparent, and the relative position of the centromere may be determined more precisely than in the root-tip metaphase figures.

The structure of the B chromosome bivalent in the pachytene stage of the meiotic prophase has been described and illustrated by McCLINTOCK (1933). Her figure 2 (p. 194) shows very clearly the characteristic appearance of the long arm of the B chromosome with the small, heterochromatic knob adjacent to the centromere, the adjoining, proximal, euchromatic region equivalent to about one-third the length of the chromosome, and the distal heterochromatic portion made up of several more or less separate regions. In this figure the centromere appears to be truly terminal, but the asymmetry of the centric region indicates the presence of a diminutive short arm folded back against the proximal knob adjacent to the centromere in the long arm.

This very distinctive pachytene structure of the typical B-chromosome is unlike that of any region of equal length in any of the A chromosomes ordinarily present in existing types of maize. This conclusion has been reached after a very critical survey of the meoitic prophase morphology in well over fifty varieties of maize, including examples of flour, flint, dent, pop, and sweet corn, a survey that was conducted primarily to throw light on the origin of the B chromosomes. This does not mean that there may not be in existence today types of maize containing an A chromosome or segment thereof that is identical with the B chromosome. However, the author's observations and in addition the very extensive observations of LONGLEY (1938) on the chromosomes of maize from North American Indians, together with his more recent survey of knob positions (LONGLEY 1939), have failed to reveal the presence of an arm of any one of the typical A chromosomes that resembles the B chromosome with respect to the form and relative positions of the euchromatic and heterochromatic parts of the chromosome. LONGLEY's studies included 41 varieties of corn from the United States and Mexico, and in addition 33 strains of Indian maize from

various regions of the United States, in many of which B chromosomes were prevalent.

Noteworthy in this connection is the fact that the heterochromatic knob-like regions of the B chromosomes are nearer the centromere than are any of the knob positions in the A chromosomes. In his extensive survey of knob positions in the A chromosomes, LONGLEY failed to find any knobs in proximity to the centromeres. They were found to occur most frequently at an appreciable distance from the centromere. Obviously, the B chromosome differs from the A chromosomes with respect to this knob-centromere relationship, since the typical B has a small knob next to the centromere, and its distal heterochromatic region is nearer the centromere than are the similar regions of the A chromosomes. The form assumed by the heterochromatin in the B's is rather different from the varied shapes of the knobs in the A chromosomes. However, both seem to have the same fundamental character.

There are very few regions near the centromeres of the typical A chromosomes that are even remotely comparable to the heterochromatic regions of the B's. The long arm of chromosome 7 near the centromere is more densely chromatic than similar regions of the other chromosomes, as is the proximal portion of the short arm of 9 in some stocks; but neither of these regions resembles very closely any portion of the typical B chromosome. The extra segment attached to the long arm of chromosome 10, discovered by Longley in certain stocks of Indian maize, is largely heterochromatic. But it is definitely unlike the B chromosome in appearance. However, it apparently has no pronounced phenotypic effect and in this respect may be said to resemble the individual B chromosome.

Although the studies of the pachytene morphology of the A chromosomes have failed to reveal any very close similarity between the B's and any one region of comparable length among the A's, they have shown that there is considerable diversity of form among the A chromosomes. Therefore it is not improbable that further studies of a similar character may yield valuable clues to the origin of the B chromosomes. In a search for new chromosome types from which the B's may have originated, the progenitors of present-day varieties of flint and sweet corn and other types in which B chromosomes are prevalent should be sought. Hybrids between diverse types of maize, as well as the intergeneric hybrids of Zea, Euchlaena, and Tripsacum should be subjected to further study. For it is possible that the euchromatin and heterochromatin comprising the B chromosomes may have become detached from some one or more of the A chromosomes and subsequently established as an autonomous chromosome following the hybridization of types that differed with respect to certain specific segments of their chromosomes.

In an elaboration of their hypothesis that Euchlaena originated from the natural hybridization of Zea and Tripsacum, MANGELSDORF and REEVES (1939, p. 203 et seq.) suggested that much of the extreme diversity of existing types of maize is to be attributed to contamination by Tripsacum by way of Euchlaena and that the knobs on the chromosomes of both maize and teosinte came originally from Tripsacum. If the knobs of the maize chromosomes actually originated from Tripsacum, it is logical to assume that the B chromosomes, or at least their heterochromatin portions, also originated from Tripsacum, since the heterochromatin of the B's and the knobs of the A's appear to be identical with respect to their general appearance, behavior in the mitotic cycle, and response to various staining reactions. On this assumption the knobless types of maize, especially those from the Andean region of South America, which according to the views of MANGELSDORF and REEVES have not been contaminated by Tripsacum, would not be expected to contain B chromosomes. However, the B's apparently are as prevalent in these essentially knobless South American types of maize as they are in Central and North American maize (LONGLEY unpublished). Furthermore, B chromosomes are conspicuously lacking in Guatemalian corn which has many knobs and shows much external evidence of Tripsacum contamination, according to unpublished observations of MANGELSDORF and REEVES.

There is no cytological evidence from the studies of MANGELSDORF and REEVES (1939) that the B chromosomes resulted from the hybridizing of Zea and Tripsacum, but these workers have presented evidence of incomplete homology among the chromosomes of these genera as well as irregularities in synapsis that might conceivably produce supernumerary fragments like the B chromosomes. The fact that the B chromosomes ordinarily do not synapse with any of the A chromosomes suggests they may have originated in remote times, which would fit in nicely with the suggestion of MANGELSDORF and REEVES that maize contains an admixture of Tripsacum chromatin from long ago. But synaptic behavior alone cannot be considered as definite proof of this assumption, since certain regions of a chromosome in the absence of other regions normally present might exhibit little or no synaptic attraction.

BREEDING BEHAVIOR OF THE B CHROMOSOMES

Very little has been published on the breeding behavior of the B chromosomes. From his pioneer studies on chromosome number in Zea, KUWADA (1911, 1915) concluded that the somatic number 24 was the base number since it was the prevalent number in varieties of sweet corn that he first examined. Later (1919) this view was abandoned after it was recognized that the more prevalent number was 20, especially in starchy maize, and

that the progeny of plants with more than 20 had variable numbers of chromosomes. However, KUWADA made no extensive determinations of chromosome numbers in the progenies of plants with different numbers of the supernumerary or B chromosomes.

LONGLEY (1927) determined the number of B chromosomes in reciprocal crosses of plants with 0-B and 1-B chromosomes, and he also intercrossed plants with one and two B's. The results indicated that the B's were transmitted freely by both male and female gametes. Furthermore, numbers higher than would be expected by gametic recombinations of the parental numbers were obtained not infrequently. This was attributed to non-disjunction in the second meiotic anaphase and the subsequent functioning of the exceptional gametes thus produced. Reciprocal crosses of 0-B and 1-B plants were also studied by POWERS and DAHL (1937). They observed that when the seed parent carried the extra B chromosome, it appeared in approximately half of the progeny, but when transmitted by the pollen parent, B chromosomes were present in less than one-third of the progeny. These results were interpreted to mean that there had been selection against pollen carrying the extra chromosome, as it was observed to assort at random in the first meiotic mitosis and divide regularly in the second division without being eliminated in the cytoplasm, except very rarely. These workers concluded that the extra chromosome was not wholly inert genetically since it affected the functional activity of the pollen. However, their results were not in agreement with those of LONGLEY, nor are they in agreement with those reported here. Recently DARLINGTON and UPCOTT (1941) reported on the frequency distributions of B chromosomes in the progenies of plants with from three to six B's. From their own data which involved a total of 31 plants from three progenies, and from LONGLEY's data on the frequencies of B chromosomes in the progenies of plants with one and two B's, they concluded that there was no evidence of the selective elimination of gametes in the transmission of the B chromosomes.

The data on the transmission of the B chromosomes, which are presented in tables 1–3, were accumulated over a period of years in connection with various cytogenetic studies of their characteristics. The Black Mexican and Golden Bantam varieties of sweet corn, and various genetic cultures in which B chromosomes were prevalent (RANDOLPH 1928a), were the sources of the stocks used in most of these studies. The breeding behavior of the B chromosomes and their appearance as well were the same, irrespective of their source, and no attempt was made to keep the different stocks separate. In fact they were often purposely intercrossed in order to utilize the resultant hybrid vigor in maintaining the stocks in good condition.

The earlier counts were made from sporocyte material. Later rapid and much simplified methods of handling root tips in the paraffin method and

TABLE 1

Frequency distributions of B chromosomes in the selfed progeny of plants with from 1 to 20 B chromosomes.

PARENT NOS. OF B CHRS.	B CHROMOSOME FREQUENCIES																								n	MEAN	S.D
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25			
1	14	17	6	6																							43	1.09±0.16	1.02
2	11	57	44	48	5	3																					168	1.93±0.08	1.08
3	3	6	21	19	10	8	4	2																			73	3.06±0.19	1.59
4	1	11	27	10	32	13	10	1																			105	3.38±0.15	1.57
5		2	4	8	9	5	7	6	3		1	2															47	5.00±0.35	2.40
6			2	10	10	15	10	5	3	2	4		1	2													64	5.69±0.32	2.53
7		1	5	7	30	16	15	13	4	3																	94	5.02±0.18	1.70
8			1	3	7	4	9	10	14	6	9	4	1	2													70	7.44±0.30	2.49
9							3	5	2	6	3	3	1	1													24	8.79±0.40	1.96
10					2	4	8	8	23	15	14	6	4	3		1											88	8.63±0.23	2.11
11						3	4	8	12	15	11	9	18	10	7	3	6	1		2							109	10.78±0.29	3.03
12			1			3	6	3	5	5	10	2	4	3	1	1	1										45	9.18±0.44	2.93
15								1	1		2	2	1		2		1	3		1							14	13.07±1.00	3.75
16									1	2	6	3	5	4	6	4	6	2	3	2	3	2	1	1			51	14.59±0.53	3.77
17											1	4	6	4	4	1	7	4	2	2	5	1		5			46	16.07±0.57	3.84
20											2		1			1			1		1	1			1		8	16.38±1.96	5.53
																										Total	1049		

a procedure for obtaining more reliable results with the crystal violet stain were perfected (RANDOLPH 1935, 1940) and were used for most of the counts that have been made in recent years. In studying the transmission of the B's, the sporocyte counts furnish a somewhat more reliable index of the chromosome numbers involved in a particular cross, since there is occasional non-correspondence between seedling root-tip counts and the sporocyte counts of the same plant, due to irregularities in the mitotic division of the B chromosomes. For the same reason, seedling counts furnish a more reliable index of the numbers of B's transmitted to a given progeny. Fortunately, non-correspondence of seedling and sporocyte counts is of rare occurrence (RANDOLPH 1928a) and is therefore an infrequent source of error. Because of the much greater ease with which they could be procured, seedling counts were ordinarily utilized in studying the transmission of the B chromosomes. Both seedling and sporocyte data are included in table 2, but the data in the other tables were obtained chiefly from seedling root-tip counts.

It was relatively simple with the methods used to determine the exact chromosome number when relatively few B chromosomes were present. But it was more difficult to determine the higher numbers with the same precision, for two reasons. In the first place, the higher numbers are more difficult to determine precisely due to crowding of the B chromosomes amongst the A's and because of a certain tendency toward non-orientation of the B's on the metaphase plate. In the second place, actual non-correspondence in the number of B chromosomes in different cells of the same individual was more prevalent, as would be expected, among the plants with large numbers of them. In such cases of non-correspondence the most frequently observed number was recorded as the typical number for the plant in question.

The number of B chromosomes in the selfed progeny of plants with from one to twenty B's is shown in table 1, which includes the frequency distributions of 1049 plants whose B-chromosome numbers were determined. From these data it is apparent that B-chromosome plants did not breed true for any given number. Inconstancy of number in succeeding generations was the rule, whether the parental number was odd or even. Various numbers were represented in the populations, the mean number in the progeny of plants with few B chromosomes being approximately that of the parental plant, while for plants with larger numbers the means of the progeny were somewhat less than the parental numbers. The range of variability in the progenies increased along with the increase in the number of B chromosomes, as shown by the differences in the standard deviations of the means of the various progenies. In all the selfed progenies there were some plants with more B chromosomes than would have been expected from the

TABLE 2

Frequency distributions of plants with different numbers of B chromosomes in direct and reciprocal crosses of 0-B and B-chromosome plants.

PARENT NOS. OF B CHRS.	B CHROMOSOME FREQUENCIES																									n	MEAN	S.D.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25			
0×1	32	13	5	2																							52	0.56±0.12	0.83
1×0	46	19																									65	0.29±0.06	0.46
0×2	22		37		2																						61	1.34±0.14	1.08
2×0	8	41	3																								52	0.90±0.06	0.45
0×3	27	4	31	1	10	1																					74	1.54±0.17	1.42
3×0	3	27	19	1																							50	1.36±0.09	0.63
0×4	42		54	3	26	1	1																				127	1.83±0.14	1.53
4×0		13	27	4	2																						46	1.89±0.11	0.74
0×5	5	6	12	12	10	2																					47	2.47±0.20	1.37
0×6	3		15	2	4		4		1																		29	2.90±0.36	1.95
0×8	6	2	3	5	14	2	8	1																			41	3.51±0.32	2.03
0×15	1	2	1	3	3	1	5	2	1	5	2	3	1														30	6.40±0.62	3.38
0×19		1	1	1	2	3		1	5	2	3	2		1				1									23	7.65±0.78	3.73
0×21	4	4	7	4	8	9	17	10	14	12	9	14	11	6	4	3	2	2	1	1				1	1		144	8.36±0.37	4.47
0×23			3	2			1		3	1	3		2	1	1	4			1		1		2				25	10.96±1.21	6.06
0×25			2	1	4	3	1	3	4	2	4	7	1	3	1		2		1		1	2		1			43	10.05±0.80	5.25
																										Total	909		

chance recombination of normally reduced gametes, and numbers lower than expected also were found among the progenies with the higher number of B's.

By selecting in each succeeding generation individuals with more B chromosomes than were present in the parents, it was readily possible to multiply the number of B's to a surprisingly high level. For example, by selfing 3-B plants from the progeny of 1-B plants, individuals with five B's were procured, and when these were in turn selfed, plants with still higher numbers were obtained. Sterility and reduced viability of the plants with many B chromosomes eventually checked this cumulative process, but not until their number exceeded the number of A chromosomes. The greatest concentration of B chromosomes was obtained by intercrossing the plants containing large numbers of them (table 3).

The B chromosomes are transmitted freely by both the male and the female gametes, as shown in the reciprocal crosses between 0-B and B-chromosome plants listed in table 2. In the cross 0-B × 1-B, approximately one-third of the progeny had one or more B chromosomes irrespective of the way the cross was made. However, there were exceptional plants with two or more B's in the population derived from the 1-B plant as the pollen parent, and none appeared in the reciprocal cross.

The reciprocal crosses between plants with two, three, and four B's and plants with no B chromosomes were very different. When the B's were transmitted by the seed parent, the numbers in the progeny were in general intermediate between the parental numbers, but when they were carried by the pollen, the 0-B, 2-B, and 4-B classes were predominant. Individuals with more B chromosomes than were contributed to the cross by the B-chromosome parent appeared in many of the 0-B × B-chromosome progenies, throughout the range from low to high numbers. In these same populations there were also many plants with fewer B's than would be expected from the functioning of normally reduced gametes. An extreme example of this wide range of variation occurred in the cross 0-B × 21-B in which there were included, among the 144 plants counted, four with none, two with more than 21, as well as plants with most of the intervening numbers of B chromosomes.

The divergence between reciprocal crosses involving plants with no and those with few B chromosomes is difficult to explain. Analysis of the behavior of the B's in microsporogenesis and in the microspore division did not solve the problem. In the 1-B plants the univalent B assorted at random in the first meiotic division and was occasionally lost in the cytoplasm; it divided regularly in the second division, and one B-chromosome was present in somewhat less than half of the microspores. These observations furnished no explanation of the two and three B-chromosome plants

that were present in the progeny of the 0-B × 1-B cross, or of the 3-B plants in the selfed progeny of plants with one B chromosome.

In the 2-B plants the B's regularly formed a bivalent during the prophase of the first meiotic division, which disjoined along with the ten A bivalents in anaphase I, except for rare occurrences of desynapsis or elimination in the cytoplasm, and divided normally in the second meiotic division. From these observations, the assumption was that, with rare exceptions, the microspores and male gametes would receive a single B chromosome, and if the meiotic behavior was the same in megasporogenesis, the selfed progeny would be expected to contain almost exclusively 2-B plants. Likewise, in crosses between 0-B and 2-B plants the F_1 would be expected to consist of 1-B plants with an occasional 0-B plant. The assumption with reference to the microspore counts was correct. Among 130 microspore division figures that were counted there were 116 with one B and 14 with no B chromosomes. None had more than one B chromosome. The assumption with reference to the number of B's in the progenies was incorrect, as indicated in tables 1 and 2.

The selfed progeny of 2-B plants did not contain exclusively plants with the parental number of chromosomes; in fact only about one-fourth of the progeny had two B's, and there were many plants with more than two B's as well as a considerable number with less than two B's, a result that was not in agreement with the result to be expected from the observed numbers of B's in the microspores. Similarly in the selfed progeny of 4-B plants, there was a wide variation in the number of B's ranging from none to seven, with a standard deviation of 2.396. However, the four B's regularly formed either two bivalents or a quadrivalent followed by a normal reduction to two B's in most of the microspores. Here again there is a marked discrepancy between the observed frequencies of the B's in the progeny and the numbers to be expected from their meiotic behavior.

It is a curious fact that the results actually obtained from the crosses of 2-B and 0-B plants were in close agreement with the results that were to be expected from the microspore counts, while the observed frequencies in the 0-B × 2-B crosses were strikingly at variance with the expected frequencies. Most of the F_1 plants from the latter cross should have had one B chromosome and the remainder no B's. Actually there were no 1-B plants among 61 that were counted. There were 22 with no B's, 37 with two B's and two with four B's. Results such as these were not to be expected from the microspore counts. There was a similar close agreement between the results actually obtained and the results expected from the microspore counts in the 3-B × 0-B and the 4-B × 0-B crosses. There was the same lack of agreement in the reciprocal crosses.

Since the expected results were obtained when the B's were transmitted

by the seed parent, there is no reason to attribute the difference between the reciprocal crosses to irregularities in megasporogenesis or to somatic non-disjunction or some other irregular type of B-chromosome division in the zygote or proembryo. However, it is possible that some types of anomalous behavior of the B's in the division of the generative nucleus to form the two male nuclei were responsible for the unexpected results. Unfortunately, the distribution of the B chromosomes in this division was difficult to determine, and further studies will be required before any positive conclusions can be drawn relative to their behavior at this stage.

The frequency distributions of plants with various numbers of B chromosomes in crosses involving parents with unlike numbers are summarized in table 3. Many such crosses were studied—first the lower numbers and later the higher numbers derived from them—primarily to determine the extent to which the B chromosomes could be reduplicated. Crosses in which the sum of the parental numbers were the same are grouped in the table, the parental combinations that were most widely different being designated in each case. For example, the only combination that would give a sum of three for the parental numbers is 1-B × 2-B and it was from this combination that the data were obtained for this particular cross, as indicated in the table; but for higher numbers, such as 14, combinations ranging from 1 × 13 to 6 × 8 might have been included, but parental numbers no more unlike than in the cross 4 × 10 were actually involved.

Since there was a relatively wide variation in the number of B's in the progenies of plants with unlike numbers of B's, and numbers higher than the number contributed to the cross by the higher parent were of frequent occurrence in all but the very highest numbers, it was possible to produce stocks with large numbers of B's simply by intercrossing the plants with the higher numbers in each succeeding generation. The limit to further reduplication was approached when it became difficult to produce stocks with any appreciable number of plants having more than 22 to 24 B's. The difficulty was due to the marked reduction in the fertility and vigor of the plants with large numbers of B chromosomes.

The mean number of B chromosomes in the progenies of the intercrosses between plants with different numbers of B's (table 3) was roughly equivalent to the mean parental number when low parental numbers were involved and was progressively less as the parental numbers increased. There was also increased variability in the number of B's in the progenies as the parental numbers increased, until the higher numbers were reached. Thereafter the range of variability was reduced, due to the infrequent occurrence of the very high numbers. The population trends in these intercrosses between plants with unlike numbers of B's were similar to those obtained from selfing B-chromosome plants.

TABLE 3

Frequency distributions of B chromosomes in crosses of plants with different numbers of B's. Crosses in which the sums of the parental numbers were the same are grouped together.

SUM OF PARENT B CHRS.	RANGE IN PARENT NOS. OF B CHRS.	B CHROMOSOME FREQUENCIES																															n	MEAN	S.D.
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34			
3	1–2	7	7	12	6	1																													33	1.61±0.19	1.12
5	1–4	1	4	6	2																														13	1.69±0.24	0.86
6	2–4	1	4	6	9	4	1	2		1																									28	3.00±0.33	1.74
7	3–4		3	3	9	6	3		1																										25	3.28±0.28	1.40
9	3–6	2	6	18	14	8	6	2	1																										57	2.90±0.20	1.50
10	4–6			8	7	8	3	3	1																										30	1.63±0.26	1.43
13	5–8		1		1	5	7	3	5	1		2	1																						26	5.81±0.44	2.25
14	4–10		1	1	2	2	7	7	9	13	4	3			1																				50	6.80±0.31	2.20
16	6–10			1			2	9	7	2	5	4		2																					32	7.50±0.38	2.14
18	8–10						1		5	9	4	7	3	2	1	1	1																		34	9.32±0.37	2.17
22	8–14				1			2	1	3	8	3	3	7	2	1																			31	9.84±0.43	2.41
24	11–13						1	3	1	1	1	6		2	3		1	1																		20	10.05±0.69	3.10
25	12–13							1	2	2	4	5	6	6	3	7	4	4																		44	11.84±0.41	2.68
26	12–14							1	2	1	2	4	5	2	2	1	3																			23	10.87±0.54	2.56
27	11–16									1	2			3	2	6	2	2	1	2	1														22	13.91±0.62	2.89
30	10–20					1	1	2	7	2	4	5	7	7	7	2	2	1	1																49	10.51±0.42	2.96
32	14–18						1	2	2	9	5	5	5	6	8	3	1	3	2	3	1														56	11.45±0.46	3.44
33	13–20						2	3	8	9	13	9	3	5	3	3	1																		59	9.36±0.31	2.34
34	15–19							1	3			2	2	6		2	1	1	3				1												22	12.32±0.79	3.72
35	10–25						1		2	1	3	7	7	9	9	10	7	12	15	9	4	7	3	4	2		1								113	15.20±0.37	3.95
36	15–21								1	1	1	4	4	5	7	11	13	13	8	8	4	8	2	3	1									1	1	96	15.93±0.42	4.14
37	17–20								3	3	7	4	2	4	2	10	4	1			1	1		2											44	12.34±0.57	3.75
38	18–20								1	1				2	1	2		3	2			1														13	14.00±1.01	3.65
39	16–23									1	2		3	1	5	5	5	8	8	6	3	1	2		1		1								52	15.77±0.47	3.36
40	17–23									1	3	3	4	7	5	6	10	5	6	5	3	1	3	2	2	1										67	15.19±0.46	3.78
41	17–24									1		4	3	1	2	1	3	1	2	3	2	3		1	2											29	15.45±0.81	4.34
43	18–25																2		2	2		5		2		1				1						15	19.73±0.88	3.41
45	20–25													1	2		2	3	2	1	4	6	3							1		1				26	18.65±0.79	4.03
47	22–25										1						2	2	3	7	2	2	1	2	2	1	1	1								27	18.96±0.69	3.59
																																		Total		1136	

The maximum number of B chromosomes obtained in the crosses involving many B's was 34, and it is probable that this represents approximately the upper limit attainable in such crosses. Relatively few plants with more than 30 B's plus the normal 20 A chromosomes, appeared in the cultures listed in table 3. A certain number of additional progenies from plants with many B's, not included in the table because very few individuals were involved in any one family, contained a certain number of plants with approximately 30 B's. But when these plants with extremely high numbers of B chromosomes were selfed or sib-crossed, they produced few if any viable seed, although some of them were fairly vigorous and produced good ear shoots and at least some viable pollen. It may be possible to produce plants with more B chromosomes than those reported here, from some especially favorable combination of genotypes, but it is improbable that plants with appreciably more B's will be produced, at least in diploid A chromosome stocks.

PHENOTYPIC EFFECTS OF THE B CHROMOSOMES

The B chromosomes of maize are not essential to the normal growth and reproduction of the plant, nor is there any conclusive evidence that they have any beneficial effects. In the various types of maize in which they occur naturally, usually in relatively small numbers and in only a certain proportion of the plants, their presence can be identified only by cytological examination of the chromosomes. The B-chromosome plants possess no phenotypic characteristics by which they can be identified when relatively small numbers are present. However, their repeated reduplication causes numerous phenotypic effects that are accentuated as their multiplication progresses.

The various genetical, morphological, and physiological effects of the presence in individual plants of large numbers of B chromosomes include (a) reduction in fertility, (b) decreased vigor, (c) production of defective seeds that are frequently germless or have partially developed endosperm, (d) scarred endosperm, (e) an increase in nuclear and cell size, caused by the presence of the additional B chromosomes, and (f) variation in pollen size and an increase in the percentage of aborted pollen grains. The larger pollen grains of the plants with many B chromosomes may exceed the dimensions of the pollen produced by triploid and tetraploid maize, indicating that the increase in the number of B chromosomes effects pollen size and cell size generally, just as does the reduplication of the A genome.

These various effects become apparent gradually as the number of B chromosomes is increased. There is ordinarily some reduction in fertility in the ears of selfed plants with from ten to 15 B's; there is still more in plants with from 15 to 20 B's. The set of seed is much reduced in plants

with from 20 to 25 B's, and those with still larger numbers of B's rarely produce any appreciable amount of viable seed either when selfed or sib-crossed. More seed is obtained from reciprocal crosses of plants with few and many B's than when plants with many B's are selfed or sib-crossed. This suggests that the combinations of gametes with many B's are non-functional and result in the failure of embryo and endosperm development. However, zygotic lethal effects also contribute to the reduction in fertility of plants with large numbers of B's, as evidenced by the occurrence of inviable, aborted seeds along with undeveloped ovules and a certain number of normally developed seeds on the ears of the plants with large numbers of B chromosomes.

The absence of any pronounced selection against pollen carrying large numbers of B's, strikingly illustrated by the data presented in table 2, is a very significant characteristic of the B chromosomes, which delimits them sharply from known A-chromosome fragments. RHOADES (1936, 1940) made a detailed cytogenetic study of a supernumerary telocentric chromosome consisting of the short arm of chromosome 5. Plants hyperploid for this telocentric chromosome transmitted it to the progeny through the pollen with a frequency of less than one percent, while it was transmittted through the female gametes with a frequency of about 30 percent. The difference was attributed to unsuccessful competition with pollen carrying normal haploid gametes in the former and the absence of similar competition in the latter.

Although this telocentric A chromosome is occasionally reduplicated to form a secondary trisome consisting of two short arms of chromosome 5 attached to a median centromere, further reduplication rarely, if ever, occurs. Similarly, the primary trisomes of the A-chromosome set, in so far as they have been studied, are transmitted readily through the female gametes but less readily, if at all, through the pollen and cannot be reduplicated repeatedly. It appears, therefore, that the B chromosomes are unique among known supernumerary chromosomes or chromosome fragments of maize with respect to the readiness with which they are transmitted by both male and female gametes and the ease with which they can be reduplicated repeatedly.

The level at which the various morphological and physiological effects of the reduplicated B chromosomes is first apparent varies considerably in different stocks and even in the same stock. Some plants with from 25 to 30 B's may be nearly normal in size, while others are very weak and fail to reach maturity. Plants with fewer than 20 B's ordinarily show no appreciable reduction in vigor, but those with more than 30 were very weak, defective plants. Similarly with respect to fertility, the plants with less than 10–15 B's usually produce well-filled ears, while those with more than from

23–25 ordinarily produce relatively little viable seed, but the plants with the intermediate numbers may or may not produce very much good seed, and their relative fertility cannot be predicted in advance.

The absence of B chromosomes in most stocks of dent corn suggested that they might have a definitely deleterious effect in these stocks. Accordingly high B-chromosome plants of sweet and flint varieties were intercrossed with dent types. It was found that the B chromosomes could readily be introduced into the dent stocks with no apparently greater deleterious effects than were noted in other types of maize.

It has been definitely established from the studies reported here that the B chromosomes exert pronounced cumulative effects when present in large numbers. Whether these effects are to be attributed to what may be designated mass action of the chromosomes—that is, to the mere presence of an increased volume of chromatin in the cell, which in the case of the plants with many B chromosomes is very appreciable—or to the presence of a marked increase in the relative amount of heterochromatin, or to specific gene action, are questions of considerable theoretical interest, which can be answered only by further study. There is some evidence that the plants with many B chromosomes are more fertile in stocks with A chromosomes having relatively few knobs, suggesting that there may be a correlation between fertility and the amount of heterochromatin in the chromosome complement. The cytological observations on meiosis in plants with many B's suggest that the B's cause less disturbance of the normal behavior of the A's when few knobs are present, which may account for this difference in relative fertility.

SPECIFIC GENETIC EFFECTS OF THE B CHROMOSOMES

Since the typical B chromosome at mid-prophase of meiosis consists of an appreciable segment of euchromatin, in addition to a somewhat longer segment of heterochromatin, it seems logical to assume that it may contain the alleles of at least some active genes. Presumably these genes would be, at least in part, duplicates of genes located in the A chromosomes. In order to explore this possibility an attempt was made to test possible loci of B chromosomes for allelism with a representative group of the known mutant genes, especially those whose linkage relations were known.

The procedure in making these tests was to cross the recessive mutant to be tested with B-chromosome plants carrying its dominant allele and then determine from back-cross and F_2 data whether the presence of B chromosomes in the segregating populations disturb the expected ratios. To minimize the amount of cytological work necessary to make these tests, the original cross was made first with B-chromosome plants having five or

more B's. Three or more F_1 plants from this cross then were selected, without determining their B-chromosome number, and either back-crossed or sib-crossed. From them a segregating population of approximately 100 plants was grown. In starting with at least five B chromosomes in making the original cross, it could be assumed that one or more of the three F_1 plants would contain B chromosomes which would in turn be transmitted to its progeny (see tables 1 and 2). If there was no disturbance of the expected ratios from the cross, it was concluded that the B chromosome did not carry the dominant allele of the gene being tested.

If unusual ratios were obtained from the cross, it was repeated, using not only plants with five or more B's, but also plants with fewer B's and with no B's to serve as controls. Actual chromosome counts of the F_1 plants also were made in some cases to make certain that the segregating progenies contained B chromosomes.

The linked genes which have been tested in this manner for dominant alleles in the B chromosomes are underscored in the accompanying linkage maps (fig. 1) (see EMERSON, BEADLE, and FRASER 1935). Superimposed on these linkage maps are tentative assignments of the centromere positions for each of the ten A chromosomes. These positions are indicated by an oval drawn either with a solid or a broken line. Unpublished data generously furnished by E. G. ANDERSON, M. M. RHOADES, and C. R. BURNHAM made possible the completion of the map.

There is more definite information about the position of the centromere for some of the chromosomes than for others. The more definitely placed centromeres were drawn with a solid line; the others were drawn with a broken line. It should be emphasized that these are tentative assignments of centromere positions, serving merely to indicate with a fair degree of accuracy the arm of the chromosome in which the various genes are located. However, the centromeres occupy submedian positions in the A chromosomes and the maps are oriented with the cytologically short arm at the left in the diagram. No attempt was made in these maps to indicate the physical positions of the genes within the various arms, although the approximate physical location of a considerable number of genes has been determined.

The 46 linked genes which were tested with the B chromosomes for allelism are distributed among 17 of the 20 arms of the A chromosomes. The short arms of chromosomes 8 and 10 and the long arm of chromosome 9 have not yet been tested.

None of these genes gave disturbed ratios in combination with the B chromosomes. Deviations from the expected ratios which appeared to be significant were obtained for certain genes, but on further tests these were found to be due either to differential viability, incorrect classification, or other causes.

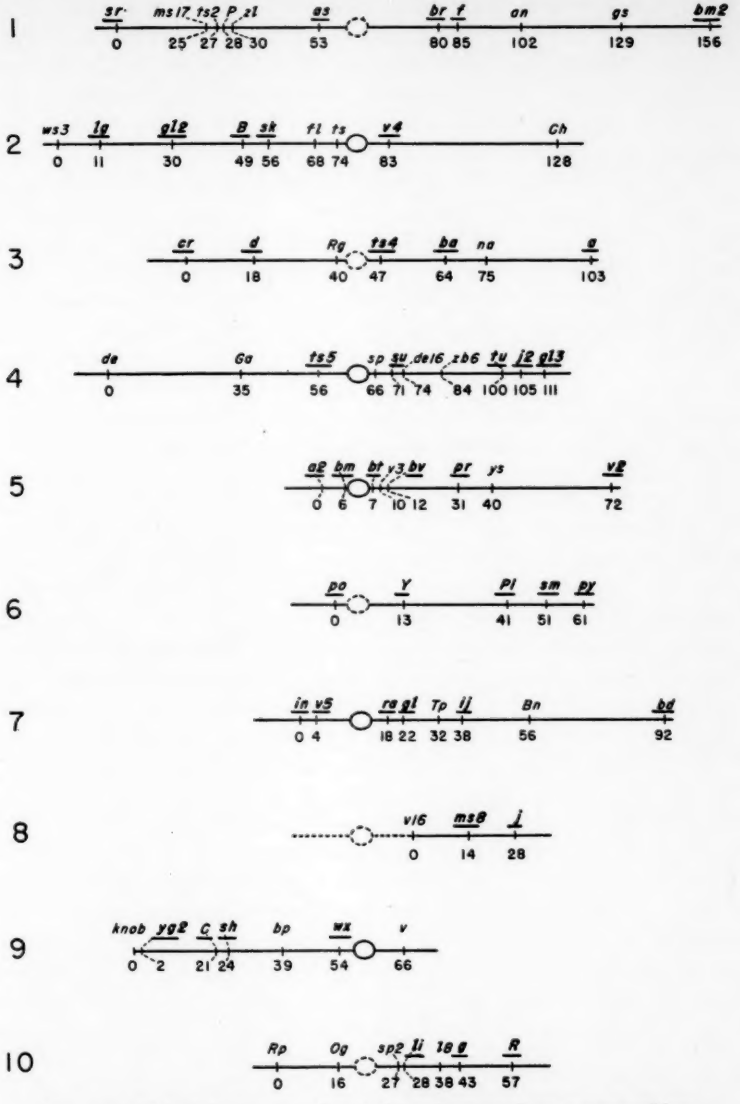

FIGURE 1.—Maize linkage maps with tentative assignments of centromere positions.

A number of unlinked genes also were tested, including *gl6*, *gl7*, *gl9*, *v17*, *ze*, *sb*, *nl* (see EMERSON, BEADLE, and FRASER for description of characters). All these genes with the exception of slit blade (*sb*) gave normal ratios with

the B chromosomes. Aberrant ratios for *sb* were found in progenies of plants known to carry B chromosomes. This suggested that they might carry the normal allele of *sb*. However, slit blade is a variable character with poor viability, and subsequent tests showed that slit blade plants carried B chromosomes, indicating that the disturbed ratios were not due to the presence of B chromosomes.

These genetic tests of B chromosomes for the presence of dominant alleles of known mutant genes by no means demonstrate that the B's are genetically impotent. The 50 genes that were tested represent less than 20 percent of the known mutant genes of maize, and there must be many more genes that have not yet been identified. However, these tests do indicate that the regions occupied by these genes are not represented in the B chromosome. In this connection, it should be mentioned that there is a possibility the B's may contain recessive genes, either alleles of certain of the genes tested, whose presence was marked by the existence of the corresponding dominant alleles in the A chromosomes, or other recessive genes with or without dominant alleles in the A chromosomes. Ordinary genetic tests would not disclose the presence of recessives in the B's whose corresponding dominant alleles were borne in the ever-present A's; but if these same recessives also were present in the A chromosomes of some stocks and not of others, this would have been noted, provided the characters involved were clearly recognizable.

The tests reported here for the existence in the B chromosomes of dominant alleles of known mutant genes involved one or more regions in 17 of the 20 arms of the normal A-chromosome complement. Presumably these arms are not represented in their entirety in the B chromosome. But one of the three remaining arms may be involved, although the cytological evidence from both length measurements and pachytene morphology very strongly suggests that the B chromosome did not originate from these particular arms—namely, the short arms of chromosomes 8 and 10 and the long arm of 9. Further cytological and genetical studies should throw additional light on the present obscurity that surrounds the problem of the origin of the B chromosomes.

B-CHROMOSOME DERIVATIVES

A notable characteristic of the B chromosomes is that they are like the A chromosomes in being susceptible to breakage, with the resultant loss of acentric segments of chromatin or rearrangement of parts. But there is this distinction—that the supernumerary B's, because they are less active genetically, can undergo a greater variety of such morphological changes than can the A chromosomes without deleterious effects on the plant, and

for the same reason their monocentric derivatives have greater survival values and can be more readily maintained in culture for further study. Over a period of years a considerable number of such B-chromosome derivatives have originated in cultures under observation, the first of these being the C chromosome that was described in 1928. Most of these derivatives of the B's have been detected in root tip figures as they were being examined for chromosome counts.

To provide a convenient system of designating the various B-chromosome derivatives, they have been grouped in four wholly arbitrary, but reasonably distinct size classes or types, based on their appearance in the somatic metaphase. These are (1) the C type that is somewhat shorter than the typical B type but definitely elongated in contrast to (2) the D type that is essentially spherical with a diameter roughly equivalent to the diameter of an ordinary chromosome, (3) the E type, also spherical but definitely smaller than the preceding type, being of approximately the same size as the undivided metaphase satellite of chromosome 6, and (4) the F type that is in turn distinctly smaller than the E type and in fact is only slightly above the lower limit of visibility of the photomicroscope. The size differences that serve to distinguish these four classes of B-chromosome derivatives are recognizable not only in the somatic metaphase but also in the diakinesis and metaphase stages of the first meiotic division. Here the individual elements are much larger than they are in the root tip figures, and when they occur as univalents, as they frequently do they are all, even including the B type, spherical or oval in shape, and size differences are readily discernible. These various types of fragments seem clearly to have originated from the B chromosomes, since they have appeared exclusively in B-chromosome stocks; and all of them, with the possible exception of certain F types, have the characteristic small knob next to the centromere, a condition that is peculiar to the B chromosome. Their frequent synapsis with the B's is further evidence of their origin from them, especially as neither they nor the B chromosomes synapse at all frequently with the A chromosomes.

It should be emphasized that this classification of the B-chromosome derivatives is one of convenience, based on size differences that are readily distinguishable in the somatic metaphase. When the various types are examined in the mid-prophase of the first meiotic division, it is apparent that qualitatively different elements of independent origin have sometimes been grouped together on the basis of this classification. The simplest but by no means the only possible mode of origin of these elements is by breakage of the B chromosome at various points, the centric portion persisting as a distinctive chromosome type while the acentric portion, if not attached in some manner to another centromere, is lost. It might be expected that

such breaks would occur at almost any point along the chromosome and thus give rise to numerous fragments of different sizes, but there is some evidence that the breaks tend to occur more frequently at certain points than at others, and to the extent that this is so the established classification into the C, D, E, and F types is a natural one. For example, the D-type fragment has arisen repeatedly in cultures under observation, apparently through the terminal deletion of most of the distal heterochromatic portion of the B chromosome, and the break seems to have occurred at about the same place each time.

The transmission of the various B-chromosome derivatives from generation to generation has been studied sufficiently to establish the fact that

TABLE 4

Frequency distributions of D chromosomes in the selfed progeny of plants with from one to fourteen D chromosomes.

PARENT NOS. OF D CHRS.	D CHROMOSOME FREQUENCIES																	n	MEAN	S.D.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
1	17	9	4															30	0.57±0.13	0.73
2	4	3	7	5														19	1.68±0.25	1.11
4	1	9	13	4	3													30	1.97±0.18	1.00
7			2	4	5	18	11	5	5	1	1	1						53	5.60±0.25	1.82
9		1		1	4	8	7	8	17	5	4	2	1		1		1	60	7.38±0.33	2.53
14					1	1	2	2	5	4	6	3	3					27	8.89±0.41	2.12
																	Total	219		

the various types can be perpetuated; and at least some of them can be accumulated in relatively large numbers much as it was possible to do with the B chromosomes themselves. Even the extremely small F chromosome, which is so minute that it is scarcely visible in somatic metaphases, and in the pachytene stage can be seen to consist merely of a centromere with an almost insignificant amount of attached chromatin, apparently behaves much as a normal chromosome throughout the life cycle. The number of F chromosomes ordinarily remained constant from the seedling stage to the sporocyte stage, and in two generations it was possible to increase their number from one to four. Such regularity in the behavior of the F "chromosome" is a striking demonstration of the ability of an essentially naked centromere to perpetuate itself.

The frequency distributions in selfed progenies of plants with various numbers of a particular D chromosome, that has been studied more extensively than other B-chromosome derivatives, are given in table 4. From these data it is apparent that this D chromosome is transmitted in much the same way as the typical B chromosome and with a similar range of variation in comparable progenies. The multiplication of this D chromo-

some has not progressed sufficiently to definitely establish the presence or absence of phenotypic effects similar to those produced with high numbers of B chromosomes. However, there is apparently some reduction in fertility among certain plants with more than ten D's. If this is established by additional crosses, it will mean that this B chromosome derivative is a more potent cause of reduced fertility than the B chromosome itself and will further suggest that the genes responsible for the effect are located in the euchromatic rather than the heterochromatic portion of the B chromosome, since the D chromosome is almost exclusively euchromatic.

SUMMARY AND CONCLUSIONS

The typical B chromosome of maize is not only appreciably shorter and has a more nearly terminal centromere than any of the normal A chromosomes, but it also has a very distinctive pachytene structure unlike that of any region of equal length in the A chromosomes. The pachytene structure of the A chromosomes from more than 50 varieties and strains including the various types of flint, dent, sweet, flour, and pop corn was compared with that of the B chromosomes in a search for an A-chromosome segment similar to the B chromosome, but none was found.

The B chromosomes are transmitted freely by both male and female gametes. However, reciprocal crosses between plants with and without B chromosomes may differ significantly with respect to the frequency distribution of plants with different numbers of B's in the progeny. That these differences were not due to differences in the meiotic behavior of the chromosomes in microsporogenesis and megasporogenesis was indicated by the direct observations in microsporogenesis and the microspore division and the results obtained when the B's were transmitted by the seed parent.

There is a wide range of variation in the number of B's in the progenies of B-chromosome plants. Plants with two and four B's do not breed true, or even approximately so, as would be expected from the observed regularity of their meiotic chromosome behavior.

Individuals with more B chromosomes than the parental numbers appeared not infrequently in the progenies of B-chromosome plants. By selecting these exceptional plants with many B chromosomes for breeding purposes, it was readily possible to multiply the B chromosomes in succeeding generations.

The number of B chromosomes was determined in (a) 1049 plants from selfed progenies of plants having from one to 20 B's. (b) 909 plants from direct and reciprocal crosses of 0-B and B-chromosome plants with from one to 25 B's, and (c) 788 plants from sib crosses of plants with different numbers of B's.

The highest number of B chromosomes in addition to the normal 20 A

chromosomes obtained in any one plant from intercrossing plants with many B's was 34. Many plants were obtained with from 20 to 30 B's, as well as lower numbers, but there were very few with more than 30 B's.

The B chromosomes are not essential to the normal growth and reproduction of the plant, nor is there any conclusive evidence that they have any beneficial effects. However, their repeated reduplication causes numerous phenotypic effects that are accentuated as their multiplication progresses. These effects include a reduction in fertility, decrease in vigor, an increase in the size of the pollen and other reproductive structures, the production of defective and germless seeds, scarred endosperm, and an increase in cell size.

In specific tests to determine the genic constitution of the B chromosomes, the presence of B's in the segregating populations caused no significant disturbances of expected ratios in crosses involving 46 linked genes and seven unlinked genes. The linked genes that were tested were distributed among 17 of the 20 arms of the A-chromosome complement. Since these tests showed that the B chromosomes do not contain the dominant alleles of these genes, it may be concluded that none of the 17 arms containing one or more of the tested genes is represented in its entirety in the typical B chromosome. The cytological evidence from comparative length measurements and pachytene morphology indicates that the remaining three arms—namely, the short arms of chromosomes 8 and 10 and the long arm of 9—are so dissimilar to the B chromosome that it is highly improbable that they were directly involved in its origin.

Derivatives of the B chromosomes induced by breakages, to which the B's as well as the A's are susceptible, include types progressively smaller than the typical B's. These derivatives were designated for convenience C, D, E, and F chromosomes, the classification of these types being based on size differences readily distinguishable in the somatic metaphase.

These C, D, E, and F chromosomes were perpetuated from generation to generation and in certain instances were multiplied in much the same way that the B chromosomes were.

Evidence is presented from observations on plants with large numbers of D chromosomes suggesting that the various genetic effects of large numbers of B's may be due to genes located in the euchromatic rather than in the heterochromatic region of the B chromosome.

The origin of the B chromosome is still shrouded in obscurity, but it is clear from the data presented here that its mode of origin was probably not as simple as has been assumed by various workers.

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SPONTANEOUS CHROMOSOME ABERRATIONS IN TRIPLOID TRADESCANTIA HYBRIDS

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IT HAS recently been found that chromosome aberrations of the types induced by various kinds of radiations occur spontaneously during the development of the pollen grains in diploid species and especially in species hybrids of Tradescantia (GILES 1940). The present studies have shown that the frequency of these aberrations is even higher in triploid Tradescantia hybrids. Furthermore, it has been found that another class of aberrations is also present at mitosis in the pollen grains of the polyploids—those types resulting from chromosome behavior at meiosis. To aid in interpreting and distinguishing this latter class of aberrations, rather extensive observations of meiotic chromosome behavior were made, and these have already been reported in an earlier study (GILES 1941). The present paper will discuss the types and frequencies of aberrations at the first post-meiotic mitosis in the pollen grains of the triploid hybrids and in their diploid and tetraploid parents. Certain observations of aberrations in the somatic (root-tip) mitoses of these plants will also be reported.

The plants used in this investigation were a diploid *Tradescantia paludosa* Anders. & Woodson ($2X = 12$), an autotetraploid *T. canaliculata* Raf. ($4X = 24$), and several triploid hybrids (all $3X = 18$) resulting from a cross of these two individuals, the diploid being used as the female parent. A further discussion of the plants used is given in the paper previously mentioned (GILES 1941). Aceto-carmine smears were used to study the pollen grain mitoses, and Feulgen smears for the root-tip mitoses.

GENERAL DESCRIPTION OF MITOSIS IN THE POLLEN GRAINS

Mitosis in the microspores of the diploid *T. paludosa* is very regular. The normal course of this division has been described by SAX and EDMONDS (1933). Only four cells with seven chromosomes, as a result of non-disjunction at meiosis, were noted in the 1,734 studied. The percentage of visibly normal pollen as recorded at the microspore diviesion was high—93 percent.

In the tetraploids, although there were no irregularities in division in most of the microspores, mitosis was not so regular as in the diploids. The orientation of the spindle axis was not always restricted to the short axis of the pollen grain (actually the long axis of the cytoplasmic mass), as is

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normally the condition, but in some cases the metaphase plate was oblique or parallel to this axis. The number of chromosomes in the pollen grains varied from ten to 14, 71.5 percent of the grains having 12 chromosomes. The percentage of visibly normal pollen at the microspore division was 71.0 percent, which is similar to that observed in other autotetraploid Tradescantias (ANDERSON and SAX 1936).

In the triploid hybrids the divisions in the pollen grains were very irregular. In a high percentage of cases the orientation of the spindle was abnormal. Also differences in the degree of contraction of the chromosomes at metaphase in different microspores were common, indicating a disturbance of the normal spiralization cycle of the chromosomes. Occasionally somatic non-disjunction was noted at anaphase, two chromatids of a divided chromosome having gone to one pole. A few cases of extreme asymmetry of the spindle, resulting in a monocentric mitosis with all the chromosomes going to one pole at anaphase were noted (fig. A). Numerous pollen grains were observed in which the tube nucleus had undergone a division. All of these abnormal events appear to be associated with the unbalanced condition of the genome in most of the spores, which is a result of both the hybrid and triploid nature of the plants. Such deviations from normal at the pollen-grain mitosis have been noted before in triploid Tradescantias (SAX 1937) and have been discussed in some detail for these and other plants in a recent paper by UPCOTT (1939).

Very little good pollen is formed in these triploids. The percentage of visibly normal grains as recorded at the microspore division ranged from 30.2 to 19.5 percent. In making these counts, all grains containing stainable cytoplasm and nucleus were counted as normals. Actually many were not well differentiated and probably would not function. Artificial germination tests indicate very few functional grains. The high percentage of abortive microspores in the triploids made it very difficult to obtain large numbers of mitoses. An average slide usually had less than 30 microspores with nuclei at metaphase or anaphase, whereas an average slide of the diploid usually had at least 200 such cells.

The frequencies of the various chromosome numbers recorded in triploid pollen grains are presented in table 1. This distribution is compared with the theoretical distribution expected at the microspore division if chromosome distribution at meiosis is at random and if there is no differential abortion of grains containing certain numbers up to the time of this division. The deviation from randomness on this basis, according to the χ^2 test, is certainly significant. To begin with, however, it is obvious that the expected distribution will not be obtained at the microspore division due to lagging and loss of chromosomes at meiosis. This should give a skewed frequency distribution with a peak at some point below nine. For autotrip-

loid Tradescantias studied previously this has been the case (*cf.* UPCOTT and PHILIP 1939). In the present plants, however, the curve is skewed in the opposite direction, the mode being at ten instead of nine. And we actually should expect in the present case an even greater skewness toward the lower chromosome numbers than was obtained with the autotriploids since the amount of lagging in the hybrids is much greater than in the non-hybrid plants. The only explanation seems to be that we have a differential abortion of pollen grains up to the time of the microspore divi-

TABLE 1

Chromosome numbers in the microspores of the triploid hybrids between T. paludosa and T. canaliculata.

PLANT NO.	NO. OF MICROSPORES CONTAINING INDICATED NO. OF CHROMOSOMES								TOTALS	
	6	7	8	9	10	11	12	13		
A	3	39	109	123	130	23	9	1	437	
B	—	12	44	88	76	23	2	—	245	
D	—	3	22	85	95	17	2	—	224	
F	3	9	40	61	98	40	4	1	256	
5	—	15	42	69	80	33	5	1	245	
1	4	32	100	243	254	42	1	—	676	
Total (obs.)	10	110	357	669	733	178	23	3*	2083	Mean 9.28
Totals (theoret.)	32.4	195.1	487.6	649.8	487.6	195.1	32.4			9.0

* This column omitted in calculating χ^2.
$\chi^2 = 216.0$
D. F. = 6
P = <0.01

sion; those microspores with the lower chromosome numbers being more inviable than those with the higher. It seems probable that this is related to the very high number of inversion bridges and other aberrations at meiosis resulting in deficiencies, as described in the paper dealing with chromosome behavior at meiosis (GILES 1941). The occurrence of these abnormalities in such a high percentage of microsporocytes results in a greater probability that those microspores having the higher numbers will contain an entire haploid set and consequently will be able to function in development through the division stage. A similar condition seems to exist in triploid tulips having a high frequency of inversion bridges at meiosis (*cf.* UPCOTT and PHILIP 1939). The unexpected recovery in triploid Tradescantias of microspores with 13 chromosomes indicates that the distribution at meiosis is occasionally very unequal.

POST-MEIOTIC CHROMOSOME ABERRATIONS OBSERVED AT THE MICROSPORE DIVISION

Types

Some of the principal chromosome configurations resulting from spontaneous structural changes during microspore development have been described and diagrammed in an earlier paper (GILES 1940). An even more complete description of aberrations, induced in the microspore chromosomes by X-rays, is given by SAX (1940). Actually it should be possible to find in untreated material all the types which can be induced by X-ray breakage of chromosomes and subsequent fusion of broken ends. As a matter of fact, almost all the types attributed to one or two breaks in the X-ray experiments have been found occurring spontaneously. These include three types of chromatid aberrations, which are the result of breaks during prophase after the chromosomes become effectively split, and three types of chromosome aberrations, which are produced by breaks during the resting stage when the chromosomes are effectively single. Two types of the chromatid breaks involve only a single chromosome—one results from a break in a single chromatid, producing a terminal deletion (fig. B); the other is the result of a break in the two sister chromatids of a single chromosome at the same locus with subsequent fusion of adjacent ends to give a dicentric and an acentric fragment (fig. C, D). Chromatid exchanges result from breaks in the chromatids of two separate chromosomes with subsequent fusion of broken ends to produce a new association (fig. E). Another possible chromatid exchange, producing a ring, has not been found.

Of the recognizable chromosome breaks involving a single chromosome the most frequent type is the one-break aberration producing a terminal deletion, which, following chromosome division results in two shortened chromosomes and two acentric rod fragments at metaphase (fig. F). The other type depends on single breaks in separate arms resulting in a ring and acentric fragment. Only one ring of this sort has been observed (fig. G). Of the types of breakage involving two independent chromosomes only one can be clearly recognized in all cases. Following the breakage, fusion of broken ends may result in an exchange of terminal segments. This type has not been scored because all such exchanges cannot be recognized. However, fusions of broken ends which give rise to a dicentric chromosome and an acentric fragment are recognizable in all cases. In table 2 these have been designated chromos me dicentrics. Occasional intercalary ring chromosome deletions have been noted, but these have not been included in the data.

Frequencies

The observed frequencies of the main types of recognizable spontaneous

aberrations occurring in the six triploid plants and in the diploid and tetraploid parents are presented in table 2. In calculating the number of breaks which have occurred (last column, table 2) to give rise to the observed configurations, terminal deletions of chromatids and chromosomes, and chromatid dicentrics have been considered to have arisen following a single break, whereas the exchange types of configurations involving two chromosomes or chromatids of two chromosomes have been considered to have arisen following two independent breaks. This method conforms to that used in previous publications for calculating the numbers of breaks produced by X-ray treatment.

It is clear that the aberrations resulting from a single break (columns 1, 3, 5) are much more frequent than those resulting from two independent

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EXPLANATION OF PLATE I

PLATE I. Figures A–T. Chromosome aberrations in a diploid, a tetraploid, and hybrid triploid Tradescantias. Figs. A–J illustrate aberrations resulting from spontaneous breaks during the post-meiotic development of the pollen grain; Figs. K–P illustrate aberrations resulting from chromosome behavior at meiosis. All photomicrographs ×1000, reduced one-half in reproduction.

FIGURE A.—Monopolar mitosis in triploid. Chromatid deletion to left.

FIGURE B.—Terminal chromatid deletion in triploid.

FIGURE C.—Dicentric chromatid bridge and fragment in triploid.

FIGURE D.—Dicentric chromatid bridge and fragment in tetraploid.

FIGURE E.—Chromatid exchange break (upper center) and chromosome deletion (to right) in triploid.

FIGURE F.—Chromosome deletion in diploid.

FIGURE G.—Ring chromosome with acentric fragments in triploid.

FIGURE H.—Dicentric chromosome and acentric fragments (exchange break) in triploid; a small micronucleus to right.

FIGURE I.—Two long acentric fragments resulting from a chromosome exchange break producing a dicentric with centromeres too close to be distinguishable. Triploid.

FIGURE J.—Telocentric chromosome arm produced by a chromosome deletion; acentric fragments below; in diploid.

FIGURE K.—Dicentric chromatid bridge without fragment (from meiosis) in triploid. One centric fragment at each pole.

FIGURE L.—Dicentric chromatid bridge without fragment (from meiosis) in tetraploid.

FIGURE M.—Dicentric chromosome resulting from non-disjunction of an inversion bridge dicentric at meiosis in triploid.

FIGURE N.—Chromosome with a shortened arm in a triploid; centric, but no acentric fragments present.

FIGURE O.—Interlocked ring chromosome in a triploid; no acentric fragment.

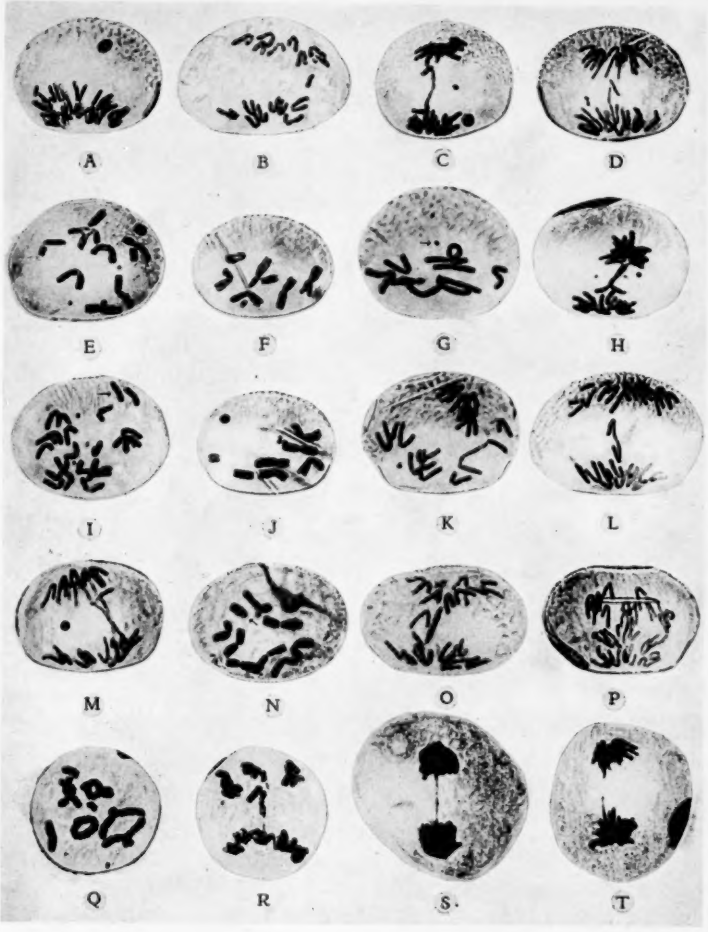
FIGURE P.—Freely separating ring chromosome with no acentric fragment; in tetraploid.

FIGURE Q.—Spontaneous chromosome break visible at meiosis in a diploid hybrid. (Photograph kindly furnished by Dr. C. P. Swanson.) Centric fragment chromosome in center.

FIGURE R.—Unequal breakage of an inversion cross-over bridge at anaphase I of meiosis in diploid.

FIGURE S.—Chromatid dicentric bridge and accompanying acentric fragment (out of focus to left) resulting from a spontaneous exchange between sister chromatids in a root tip cell of tetraploid.

FIGURE T.—Secondary chromatid bridge without acentric fragment in a root tip cell of diploid.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breaks (columns 2, 4, 6). (In the case of the chromatid dicentrics, we have followed the interpretation of SAX (1940) who considers that the breakage and subsequent fusion occurs after the effective chromosome division. This would mean that the chromatid dicentrics are actually the result of the breakage of two sister chromatids at the same locus. Consequently, on

TABLE 2

Types and frequencies of post-meiotic spontaneous chromosome aberrations found in the pollen grains of Tradescantia.

PLANTS	TOTAL CHROS.	CHROMATID			CHROMOSOME			%* BREAKS
		1 DI-CENTRIC	2 EX-CHANGE	3 TERMINAL DELE-TION	4 DI-CENTRIC (EX-CHANGE)	5 TERMINAL DELE-TION	6 RING	
3n hybrids (av. 6 plants)	19,447	34	4	21	18	37	1	.71
% aberration types		.175	.020	.108	.093	.190	.005	
T. paludosa (2n)	10,408	2	—	—	—	4	—	.06
% aberration types		.019	—	—	—	.038	—	
T. canaliculata (4n)	10,024	2	—	—	3	3	—	.11
% aberration types		.020	—	—	.030	.030	—	

* Exchange types (2, 4, 6) counted as two breaks.

this interpretation, although two breaks are necessary to produce this type, it is termed a one-break aberration by analogy with the X-ray results where it is labeled a one-hit aberration and shows a linear increase with dosage. It is possible, however, that an alternative explanation proposed in a recent paper by DARLINGTON and UPCOTT (1941) to account for the chromatid dicentrics is correct. These workers consider that chromatid dicentrics are the result of single breaks during the resting stage followed by sister reunion of chromatids after chromosome division. It may be that these two interpretations are not so greatly different as they appear to be, for it may be that most of the spontaneous breaks resulting in chromatid dicentrics occur very close to the actual time of chromosome division, at least in Tradescantia.)

One rather unexpected result of this study was the finding of such a rela-

tively large number of two-break or exchange aberrations. In the previous investigation of diploid hybrids already cited, such types were very rare, only one dicentric chromosome being noted in more than 70,000 chromosomes observed. In the case of the triploids, however, 23 two-break aberrations (exchanges) were recorded in a total of 19,447 chromosomes. This condition is apparently due to the fact that the actual frequency of spontaneous chromosome breakage in the triploid hybrids is considerably greater than in the diploids. The average for the six hybrid plants is 0.71 percent. To compare this figure with that obtained for the diploids we must omit the chromatid deletions, which were not recorded in the earlier study. The value for the remaining types of aberrations in the triploids is then 0.60 percent, which is five times as high as the value of 0.12 percent for the diploid hybrids. That a considerable part of the increase is due to the two-break aberrations is shown by the fact that the frequency of one-break aberrations in the triploids is only 3.2 times as great as in the diploids. This agrees with the X-ray experiments where it is found that an increase in the initial frequency of breaks causes a greater than linear increase in the percentage of exchanges (the exchanges increase exponentially with increasing dosage).

As was found to be the case for the spontaneous aberrations in the diploids examined previously, the frequency of chromosome deletions when compared with chromatid dicentrics is much greater than is expected from the X-ray data. In the present study the two types appear to occur with about equal frequency. It is also noteworthy that chromosome exchanges are over four times as frequent as chromatid exchanges. In the case of X-ray induced breaks, however, if the effect of a given dose is studied at prophase and then at the resting stage, the former stage is found to be much more sensitive in terms of recognizable aberrations, since about twice as many chromatid as chromosome exchanges are produced. These differences in the relative frequencies of aberration types which have been found on comparing spontaneous and X-ray induced aberrations apparently result from the fact that the resting stage is considerably longer than is the prophase, for it is during this former period that effective spontaneous breaks will produce chromosome rather than chromatid aberrations.

In the present study we are able to compare directly the frequency of spontaneous aberrations in the diploid and tetraploid pure species and in the resulting triploid F_1 hybrids. In the diploid, the frequency is 0.06 percent, which agrees quite well with that of 0.04 percent recorded in other diploids studied earlier. For the tetraploid, the frequency is 0.11 percent. In the triploid hybrids, the frequencies are much higher, ranging from 0.54 to 0.97 with an average of 0.71 percent. This means that, on the basis of all

types of recorded aberrations, the frequency of spontaneous chromosome breaks in the triploid hybrids is over six times as great as in the tetraploid parent, and almost 12 times that in the diploid parent. This increase in the frequency of spontaneous chromosome aberrations following hybridization agrees with the earlier results on the diploid Tradescantias, where the average percentage of breaks in the hybrids was about three times that of the pure species. The break frequency in the present hybrid triploids, however, is almost six times as great again as that in the diploid hybrids. Apparently this striking increase in the break frequency in the triploid hybrids must be due to the even more abnormal conditions in their microspores than in the hybrid diploid microspores. Not only have the microspores of the triploids been produced by hybrids, but they also possess in most cases numerically unbalanced chromosome complements. As has been mentioned previously, one of the striking results of these conditions is the frequent disturbance of the normal synchronization of the chromosome coiling cycle and of the mitotic division in the microspore. Since the same localization of breaks in the regions proximal to the centromere occurs in the triploids as was observed earlier in the diploid hybrids, this upset in the coiling cycle lends considerable support to the suggestion made in the previous study that the higher frequency of spontaneous chromosome breaks following hybridization may be due to recombination of genetic factors causing a disturbance of the normal timing of the coiling cycle of the chromosomes.

The high frequency of breaks in the triploids is further illustrated by the occurrence of microspores containing more than one aberration. Only a few of these were noted, and as a rule they contained only two one-break aberrations. However, three cases were observed where three breaks in the same cell had occurred. One of these, with a chromatid exchange and a chromosome deletion, is shown in figure E.

Unusual aberration types

Certain unusual cases noted during the course of the observation may now be mentioned. Occasional cells were observed in which a general fragmentation of the chromosomes had taken place. These cells were not included in the data, but it seems quite probable that occasionally excessive unbalance may cause extensive chromosome breakage. In other microspores all degrees of breakage were noted from achromatic spots on a chromosome to fine chromatic threads connecting broken ends. Only complete breaks were scored, however. Very infrequently the broken ends of the two acentric fragments resulting from breaks in sister chromatids may fail to fuse, even though the other two broken ends have fused to produce the usual dicentric bridge. The results of such behavior cannot always be dis-

tinguished with certainty from cells in which single acentric fragments produced by inversion crossing-over at meiosis have been included in the nucleus through both divisions and, having divided, are present at the microspore division as two acentric rods. Such behavior appears to be very rare in Tradescantia, however, and only three fairly certain cases were observed.

The origin of aberrations of the type shown in figure I where two very long acentric fragments only are present becomes clear as a result of other observations on spontaneous and X-ray induced chromosome dicentrics. If each of the two breaks is very close to the centromere, the resulting dicentric cannot be distinguished from a normal chromosome, and two very long acentric fragments result. Both centromeres of such dicentrics apparently always go to the same pole, and the dicentrics have all the appearances of being perfectly functional chromosomes.

One interesting case was noted in the diploid of the origin of a chromosome with an apparently terminal centromere. In this case a chromosome break had occurred just at the edge of the centromere, and the resulting acentric fragment and the telocentric chromosome arm have divided (fig. J). Such a chromosome with a terminal centromere has been shown by the work of RHOADES (1940) not to be stable in maize.

CHROMOSOME ABERRATIONS IN THE MICROSPORE RESULTING FROM CHROMOSOME BEHAVIOR AT MEIOSIS

Types

The three commonest types of chromosome aberrations resulting from meiotic irregularities and recovered at the first post-meiotic division have been described in an earlier paper (GILES 1940). They may be distinguished from the spontaneous break types by the absence of accompanying acentric fragments. These are the chromatid dicentrics resulting from the fusion of broken ends of sister half-chromatids following the breaking of inversion bridges at meiosis (fig. K, L); chromosome dicentrics resulting from the passage to the same pole at both meiotic anaphases of two chromosomes connected by an inversion bridge (fig. M); and chromosomes with shortened arms, which may originate in different ways, as will be described later (fig. N). In addition to these, two other types have been noted, centric ring chromosomes (fig. O, P) and chromosomes with exceptionally long arms.

Frequencies

The frequencies of these various types of aberrations in the triploids, diploid, and tetraploid are given in table 3. For all the types shown, with

the exception of the rings for which the numbers are too small to permit a comparison, the frequencies are much higher in the triploids. Since all these aberrations are the result of aberrant behavior at meiosis, this is not surprising, as the triploids show a much greater degree of meiotic irregularity than do the diploid and tetraploid. It is also clear that in the diploids no irregularities are present in microspores having the normal haploid complement of six chromosomes. This must be due to the fact that microspores deficient for certain chromosome sections are unable to develop, since all the aberrations tabulated produce deficiencies at meiosis due to

TABLE 3

Types and frequencies of aberrations in the pollen grain of Tradescantia resulting from chromosome behavior at meiosis.

PLANT	TOTAL CHROS.	CHROMOSOME DICENTRIC	SHORTENED ARM	RING	TOTAL CHROS.	CHROMATID DICENTRIC
3n hybrids (av. 6 plants)	19,447	20	30	2	11,290	58
% aberration types		.103	.154	.010	—	.514
T. paludosa (2n)	10,408	—	2*	—	7,270	—
T. canaliculata (4n)	10,024	2	4	1	5,027	3
% aberration types		.060	.080	.020	—	.060

* In pollen grains containing six normal chromosomes.

the loss of acentric fragments. Only in occasional cases where such aberrant chromosomes are included with an entire normal set can the microspore develop. This is the case in the two microspores containing chromosomes with shortened arms. Further evidence that deficient microspores do not develop is provided by the failure to find microspore bridges resulting from meiotic bridge breakage even in hybrid diploids which have a high frequency of inversions at meiosis. In a diploid hybrid having a bridge frequency of approximately 30 percent in terms of microsporocytes at anaphase I of meiosis, no bridges were found in over 200 microspores observed in anthers in both late and early stages of division. Also, none were noted in the previously cited study on diploids. In the autotetraploid, however, where deficiencies may be covered by the presence of an extra set of chromosomes, all the abnormalities occurring in the triploid are found. As pointed out, their lower frequency in the microspores of the tetraploid is due to the more regular meiotic behavior of this plant.

Chromatid dicentric bridges

The presence of bridges at the microspore division resulting from the fusion of broken ends of sister half-chromatids following inversion bridge breakage at meiosis has been observed previously by SAX (1937) in a triploid Tradescantia and by MCCLINTOCK (1938a) in Zea. In the latter paper, considerable evidence is presented indicating that such a union of broken ends always occurs when an inversion bridge breaks at meiosis in maize. In the present study a preliminary quantitative comparison of the percentage of bridges at meiosis and at the pollen grain division suggests that such sister chromatid fusion following bridge breakage may not occur in all cases.

Dicentric chromosomes

The dicentric chromosomes without fragments at the microspore division are the result of the passage to the same pole of both centromeres of dicentric chromatids at meiosis. Such behavior may occur at anaphase I or II, and although it is undoubtedly much more common at the first meiotic division, dicentric chromatids have been observed to pass to one pole at A II. In 1015 cells of the triploids at meiosis, 30, or 2.9 percent showed a dicentric chromatid going to one pole. If orientation of the centromeres of the dicentric chromatid is at random at the next division, passage of the dicentric to one pole should occur in half the cases. Thus 0.73 percent of the microspores should possess such a dicentric chromosome. The observed percentage is approximately one percent. These dicentrics separate at the pollen grain mitosis in the same way as those resulting from spontaneous exchanges.

Chromosomes with shortened arms

The type of aberration which is next most frequent to the dicentric single bridge is that of a chromosome having a shortened arm without any accompanying acentric fragment at the microspore division (table 3, column 3). Apparently such chromosomes may arise in several ways. Possibly the most common method is as a result of crossing over in certain unusual configurations at meiosis. Such configurations are inversions (particularly those including the centromere), translocations, and duplications (see RICHARDSON, 1936, for diagrams). As pointed out in an earlier paper on chromosome behavior at meiosis in these plants (GILES 1941), we have evidence for the occasional presence of at least two and possibly all three of these types. Another method by which shortened chromosomes may originate is the occurrence of spontaneous breaks at, or previous to, meiosis. Such breaks appear to be very rare, however, and none have been observed in this survey. That they do occur, however, is shown by observations of SWANSON (unpublished) in certain diploid Tradescantia hybrids

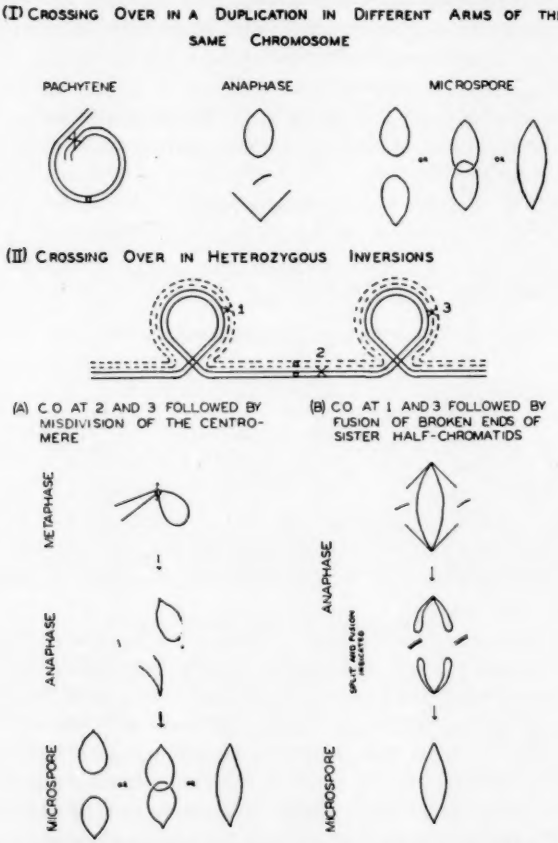
(fig. Q). A third possible method of origin of the short arm types is by the breakage of inversion bridges at meiosis. If the broken ends of such bridges do not fuse in all cases and such broken ends can heal and act as normal ends, then two shortened deficient chromosomes result. Whether such deficient chromosomes can be detected at the microspore division depends on the extent of the deficiency, since only the grosser changes in chromosome morphology are noticed. In most of the bridges resulting from inversion crossing over the deficiency is small and if the bridge breaks very near the middle, the resulting change in chromosome morphology is slight. However, breaks nearer either of the two centromeres (fig. R) produce considerably greater changes, some of which can be detected in the microspore. The several cases of chromosomes with subterminal centromeres may have arisen as a result of such unequal bridge breakage. It is not clear, however, why the complementary long-armed types are not observed more often, although they would probably not be so noticeable as the deletions.

Ring chromosomes

The last type of microspore aberration resulting from meiotic irregularities is the centric ring chromosome. As recorded in table 3, two rings were seen in the triploids and one in the tetraploid. Besides these, four others were observed in slides of the triploids not included in the data. In four of the seven cases, the two sister halves were interlocked (fig. O), and in three the two sister halves were free and separate. The two sister halves of the ring observed in the tetraploid were also free (fig. P). No double size dicentric ring, such as may be produced by X-raying in Tradescantia microspores, was seen.

Though rare, spontaneous ring chromosomes have been observed in five other genera: Crepis (NAVASHIN 1930), Zea (MCCLINTOCK 1932, 1938b), Drosophila (MORGAN 1933; STURTEVANT and BEADLE 1936; SCHULTZ and CATCHESIDE 1937), Tulipa (UPCOT 1937), Nicotiana (STINO 1940). In most of these cases the rings have apparently resulted from a spontaneous exchange between arms of the same chromosome. Only in the case of Tulipa is the situation really comparable to that described here. One of the methods of origin of rings in Drosophila, that of crossing over within an inversion in attached X chromosomes, however, is very similar in principle to what probable happens in Tradescantia. Some of the possible methods by which dicentric rings without fragments may originate are diagrammed in text-figure 1. If pairing and crossing over occur within a duplication present in both arms of a single chromosome (when the homologous regions have the same linear sequence in the chromosome as a whole), a centric ring chromosome may be produced. Such a ring chromosome should divide to give all three possible types of rings at the following

division of the microspore. The evidence for the type of pairing at meiosis necessary to produce such a ring has been cited in a previous paper (GILES 1941). Crossing over within an inversion and disparate crossing over proximal to the inversion will produce a loop chromatid. If this loop should



TEXT-FIGURE 1.—Possible methods by which centric ring chromosomes observed at the first post-meiotic mitosis in the microspore may be formed at meiosis. (For discussion see text.)

happen to be in an unoriented univalent, misdivision of the centromere at anaphase I could produce a centric ring. The occurrence of univalent bridges at anaphase I indicates that such a configuration as diagrammed is not impossible. Misdivision, however, has not been observed in the Tradescantias studied and evidently does not account for the rings observed. In such plants as tulips where it is known to occur and where inversions are

frequent, it may be responsible for some of the rings observed (UPCOTT 1937). A third method by which a ring may originate is the fusion of broken ends of sister half-chromatids following the breakage of an inversion bridge in each arm of a paired bivalent at anaphase I. Apparently such a fusion could produce only a double size dicentric ring at the microspore mitosis. Such rings have not been observed. The evidence of MCCLINTOCK (1938a) on maize indicates that the broken ends of the chromatids of a double bridge do not themselves fuse, nor does fusion of non-sister half-chromatids appear to occur. In the present case, the evidence indicates that the rings probably arise as a result of pairing in duplicated segments, rather than by the other two methods. Although we have no direct evidence for such pairing in the tetraploid, it very probably does occur, at least occasionally. The low frequency of rings in all cases is evidently due to the rare occurrence of those configurations at meiosis which will produce them.

CHROMOSOME ABERRATIONS OBSERVED DURING MITOSIS IN THE ROOT TIPS

In order to get some idea of the types and comparative frequencies of aberrations in parts of the plant other than microspores, a study was made of mitosis in the root tips of the triploids, the diploid, and the tetraploid. The results are presented in table 4. All the types of aberrations found also

TABLE 4

Types and frequencies of chromosome aberrations in the root tips of Tradescantia.

PLANT	NUMBER OF CHROMOSOMES	CHROMATID DICENTRIC (WITH FRAGMENT)	CHROMATID DICENTRIC (NO FRAGMENT)	PAIRED ACENTRIC FRAGMENTS	% ALL TYPES
3n hybrids	27,324	1	3	—	.015
2n	15,840	—	3	1	.025
4n	14,856	1	2	—	.020

occur at the microspore division, but only a few of the possible types were observed in the root tips. A chromatid dicentric with its accompanying acentric fragment (fig. S) was seen in two cells. Bridges without fragments (fig. T) were observed eight times, and a single cell with a pair of acentric fragments was seen. The chromatid dicentrics with accompanying fragments evidently resulted from spontaneous breakage and union of sister chromatids. The bridges without fragments apparently result from fusion of broken ends of previous bridges. The fact that they are relatively infrequent, however, indicates either that such fusions take place for only a few cell generations and that the ends eventually heal, or that cells containing bridges are eliminated, perhaps because such deficient cells are not able to

survive in competition with normal cells. The gradual disappearance of bridges by one of these methods in indicated by the evidence on the subsequent behavior of X-ray induced dicentrics in root tips (SAX 1940) and also by the relative infrequency of bridges in the root tips of mature plants grown from seed having a high rate of spontaneous aberrations in the seedling stage (NAVASHIN and GERASSIMOVA 1936; NICHOLS 1941).

The detection of aberrations in root tip mitoses is not so easy as in microspore mitoses. However, only anaphases in root tips were studied, when bridges between poles are obvious and lagging acentric fragments should be easily detected. Consequently, the direct comparison of spontaneous aberration frequencies in the two cases seems permissible. It is clear that the frequency of aberrations is much less in the root tips, especially of the triploids, than in the microspores. It is also clear that there is no appreciable difference among the three types of plants; actually a lower aberration frequency was observed in the triploids.

SUMMARY OF ALL ABERRATION FREQUENCIES

In order to summarize all the observations on aberration frequencies, table 5 is presented. Here it is possible to compare at a glance the results of

TABLE 5

General summary of the frequencies of all types of chromosome aberrations in the plants of Tradescantia studied.

PLANT	POLLEN GRAIN MITOSES					ROOT TIP MITOSIS	
	% BREAKS (CHRO. BASIS)	% BREAKS (CELL BASIS)	% ABERRATIONS			% ABERRATIONS	
			POST MEIOTIC	FROM MEIOSIS	TOTAL	CHRO. BASIS	CELL BASIS
Triploid hybrids (Avs. for 6 plants)	0.71	6.60	5.49	7.26	12.75	0.015	0.27
Diploid	0.06	0.35	0.35	—	0.35	0.025	0.30
Tetraploid	0.11	1.31	0.95	1.54	2.48	0.020	0.48

the studies at pollen grain and at root tip mitoses. The percentages of breaks and aberrations are given on both a chromosome and a cell basis. Comparisons have already been made of the spontaneous break frequencies on a chromosome basis. It is interesting now to consider the percentages of aberrations (not breaks) on a cell basis. For the post-meiotic types the ratio of diploid, tetraploid, and triploids is approixmately 1 to 3 to 16, the triploids having aberrations resulting from spontaneous breaks in 5.5 percent of their microspores. As was noted earlier, no aberrations resulting from meiosis are found in normal diploid microspores, but they are present

in over 1.5 percent of the tetraploid, and over seven percent of the triploid cells. If we consider the totals for all types of detectable aberrations observed at the first post-meiotic mitosis in the pollen grain, the results are even more striking. The ratio diploid: tetraploid: triploid is then approximately 1:7:36, indicating the striking increase in aberrations in the chromosomes of the pollen grains as a result of polyploidy and hybridity. Actually, in the case of the triploid hybrids, one cell in eight contains some kind of visible aberration. Since many of the possible types of structural change cannot be detected by the methods used, it seems reasonable to suppose that every cell may contain some sort of aberration. Although many of these aberrations undoubtedly inhibit the further development of the male gametophyte, numerous others must be preserved, especially in the autopolyploids, and produce structural variation in succeeding generations. It seems clear, then, that the chromosomes in the pollen grains of autopolyploids, and especially of hybrids, show an increased rate of structural change as compared with diploid pure species. In the case of the autotetraploid, this is the result primarily of the preservation of certain aberrations which are normally eliminated in the diploid. In the triploid hybrids, however, the hybrid condition itself, resulting in aberrant genetic segregation and chromosome unbalance in the pollen grains, appears to be responsible in large part for the increased amount of chromosome change. That chromosome unbalance plays some part is supported by the observations on the frequency of chromosome aberrations in the somatic (root-tip) cells. In the case of these cells, which have in all the plants normally balanced chromosome complements, the triploids do not have a higher break-frequency than their diploid and tetraploid parents.

SUMMARY

The types and frequencies of spontaneous chromosome aberrations were studied at the first post-meiotic mitosis in the pollen grains of a diploid *Tradescantia paludosa*, an autotetraploid *T. canaliculata*, and several hybrid triploids resulting from a cross of these two plants. Two general classes of aberrations are distinguishable, those resulting from chromosome behavior at meiosis and those produced by spontaneous chromosome breaks during the post-meiotic development of the pollen grains.

The aberrations resulting from chromosome behavior at meiosis, which may be distinguished in general by the absence of accompanying acentric fragments, consist of dicentric chromosomes, chromatid bridges, ring chromosomes, and chromosomes with shortened arms. The methods by which the various types originate at meiosis are discussed. These aberrations are most frequent in the triploids, which have the greatest amount of aberrant chromosome behavior at meiosis, and are entirely absent in normal haploid

pollen grains, since the meiotic irregularities producing them inhibit haploid pollen grain development.

The aberrations resulting from spontaneous chromosome breaks during post-meiotic development are similar to those induced by irradiation at this stage. They are much more frequent in the hybrid triploids than in the two parental pure species, the ratio of break frequencies in the diploid, tetraploid, hybrid triploids being 1:2:12. It seems probable that the increase in spontaneous chromosome aberrations in the triploid pollen grains results from the genetic unbalance in these cells. This conclusion is supported by the fact that spontaneous aberrations in the root-tip cells, where there is no unbalance, occur not only very infrequently, but also with approximately equal frequencies in the two parental plants and in the hybrids.

The present evidence suggests that structural chromosome changes may be found generally to originate in the male gametophyte generation with a higher frequency in hybrids than in pure species, and that such changes, plus others of similar types derived from chromosome behavior at meiosis, are preserved and may be transmitted to later generations in the pollen grains of autopolyploids.

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TESTS FOR LINKAGE IN THE GUINEA PIG*[1]

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INTRODUCTION

NO CASE of linkage has hitherto been established in the guinea pig although eight series of alleles have been tested in a rather extensive 8-factor backcross (WRIGHT 1928) and others have been tested with some of these (WRIGHT 1927; GREGORY 1928). Data can now be presented on the relations of two other series with each other and with all of the eight referred to above and on the relations of all of these with sex.

It has been obvious that none of these or any other known genes of the guinea pig show complete sex linkage, but since KOLLER and DARLINGTON (1934) found evidence in the rat that there are homologous segments in the X and Y chromosomes in which chiasmata occur and since HALDANE (1936) found indications of partial sex linkage in man, it has become necessary to examine mammalian linkage data with partial sex linkage in mind.

Finally, data will be presented on the relations of normal polydactyly.

GENES AND STOCKS

The data to be reported here involve the following genes. COLOR PATTERN: Type = self agouti *S E A*; *s* = spotting, colored spots on white ground; e^p = tortoiseshell, *e* = self yellow; *a* = non agouti. INTENSITY AND QUALITY Type = intense black and intense yellow (or "red") *C F P B*; c^k, c^d, c^r = grades of dilution, c^a = albinism; *f* = fading yellow (dark pigments affected only in *ff pp*); *p* = pink eye (dark pigments reduced, yellow unaffected); *b* = brown (brown in place of black, yellow unaffected). HAIR DIRECTION: Type = smooth *rr MM*; *R* = rough fur (at least on hind toes); *m* = modifier (*R mm* = full rough, *R Mm* = partial rough). NUMBER OF DIGITS: Type = 4 toes on front feet, 3 toes on hind feet, *px px*; *Px* = monstrous polydactyly (pollex best indicator of *Px px*; hallux occasionally present, little toes usually present, *Px Px* monstrous). Multiple factors may give normal polydactyly (four toes on hind feet) in *px px*.

The principal stocks used had the following constitutions. Genes that were involved only rarely in the present experiments are put in parentheses. Stock A—*S E A C F P B R M Px px*, hindfeet 3-toed in *px px*; Stock B—*s* e^p *a* c^k (c^r) *F p b r m px*, hind feet 3-toed; Stock C—*S* e^p *a* c^d

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f p B r m px, hind feet 3-toed; Stock D—*S e^p a C F P B r m px*, hind feet with four good digits; Stock E—*s e^p a (c^k) c^d c^r (c^a) F p B r (M) m px*, hind feet 3-toed; Stock F—*s e^p a C f P b r m px*, hind feet sometimes 4-toed (19 percent).

The 8-factor cross previously reported was between stock A (before introduction of *Px*) and stock B. These data are included here. The new data come from backcrosses (AD)D, (AE)E, (AF)F, all made in several ways with respect to sex, and F_2's $(BD)^2$ and $(CD)^2$.

POSSIBLE ERRORS OF CLASSIFICATION

There is overlapping of the amounts of white in the coat in *Ss* and *ss*. The amount of white has been estimated in terms of parts in 20 from

TABLE 1

Grades of white and yellow spotting in D, BD, and $(BD)^2$. Strain B consisted of animals with both white and yellow spotting usually well developed.

GENERATION	GRADE OF WHITE									No.
	NONE			TRACE (X)			1	2–6	7–20	
	YELLOW			YELLOW						
	X–1	2–6	7–20	X–1	2–6	7–20				
D (All *SS*)	100									47
(BD) (All *Ss*)	60.9	18.8	1.5	8.7	10.1					69
$(BD)^2$	46.4	8.8	2.6	9.4	8.6	6.0	2.3	7.6	8.3	384

drawings made in rubber stamp outlines at birth. In (AB)B, discussed previously, it was found that the backcross generation could be divided most nearly on a 1:1 basis by a cleavage between grades 1 and 2 (that is, at 7.5 percent white) in males and between grades 4 and 5 (22.5 percent) in females. Strain D has been shown to have modifiers that reduce severely the amount of white in *Ss* and *ss* (WRIGHT and CHASE 1936). F_1 of (BD) consisted of 81 percent self, 19 percent with a trace of white, but none of grade 1 or higher. In F_2, about 58 percent were self colored, 24 percent had a trace of white, while only 18 percent were of grades 1 to 18, with no important difference between males and females. Since the last class is significantly less than 25 percent, it is clear that the class with a trace of white must have included some of constitution *ss* as well as many *Ss*. It is very unlikely, on the basis of other experience, that any of those completely lacking white were *ss* (WRIGHT and CHASE 1936). An attempt was made to split the class with a trace of white by taking into account another effect of *s*—a tendency to increase the amount of yellow in the tortoise-shell pattern and to segregate black and yellow into well defined areas in

contrast to the intimate mixture of black and yellow hairs characteristic of $e^p e^p$ *SS* (WRIGHT 1917, 1923; CHASE 1939). Strain D consisted wholly of brindles with only a trace of yellow in most cases and never more than 5 percent. F_1 consisted largely of these types but with greater amounts of yellow in about one-fourth of those with no white and in more than half of those with traces of white. Only one animal had more than 32.5 percent yellow in the coat. In F_2 there were many more that had large amounts of yellow in the coat in the class with a trace of white, and practically all of those with more than a trace of white were of this sort. For purposes of classification, all of those with a trace of white and more than 32.5 percent yellow, as well as all with more than a trace of white, are considered to be *ss*, giving an estimated frequency of 24.2 percent *ss* in this generation. Doubtless some were misclassified, but it is not believed that estimates of linkage would be seriously affected.

In experiment (AF)F fairly satisfactory 1:1 ratios were obtained by drawing the line between *Ss* and *ss* at 2.5 percent white in the case of males and at 12.5 percent white in the case of females. Since *s* was present in stock A after introduction of *Px*, some spotted individuals (*ss*) appeared in F_1. A considerable number (128) of backcross animals came from three F_1 parents with only five to ten percent white, mated as possible *Ss*, but which turned out to be *ss*. A few others (11) came from high grade spotted that were obviously *ss*.

Table 2 shows the distribution of colors in experiment (AF)F omitting the classification into *Ss* and *ss*. Thirty backcross individuals that were necessarily $e^p e^p$ (F_1 parent tortoiseshell) are given in parentheses and are not included in the calculation of goodness of fit. Since *Ff* and *ff* cannot be distinguished in self blacks and browns, recombinations of the four pairs of alleles considered here result in only 14 distinguishable classes. The deviations from the calculated values yield $\chi^2 = 23.9$ which with 13 degrees of freedom indicates a probability of .04. The rather high χ^2 is largely due to one class, the brown-red agouti (*E A F b*). It is possible that this class is swelled somewhat at the expense of the brown-yellow agoutis (*E A f b*) and the brown-red agouti with red spotting (e^p *A F b*). Gene *F* is not completely dominant over *f*, and genotypes *Ff* and *ff* are somewhat difficult to distinguish in agoutis, especially in brown agoutis. A number of doubtful cases were resolved by breeding tests but not all. With regard to overlap with e^p *A F b*, it may be said that brindling is easily overlooked in agoutis and perhaps especially in brown-red agoutis where the effects of contrast are least. These possibilities of error must be considered in evaluating the data on linkage. In the case of *F* and *f* it is believed that the most reliable results are obtained by restricting consideration to animals with yellow spotting (e^p).

TABLE 2

The segregation of color varieties in experiment (AF)F. The numbers in parentheses refer to animals from matings in which both parents were $e^p e^p$. It is probable that there was occasional failure to distinguish E A f b and e^p A F b from E A F b. There was also segregation of genes S s, R r, M m, and Px px in this experiment.

GENOTYPE	COAT COLOR	EYE COLOR	OBSERVED NUMBER	EXPECTED NUMBER
E A F B	black-red agouti	black	25	23.7
F b	brown-red agouti	brown	43	23.7
f B	black-yellow agouti	black	26	23.7
f b	brown-yellow agouti	brown	18	23.7
E a – B	black	black	44	47.4
b	brown	brown	48	47.4
e^p *A F B*	black-red agouti and red	black	25 (3)	23.7 (3.75)
F b	brown-red agouti and red	brown	17 (4)	23.7 (3.75)
f B	black-yellow agouti and yellow	black	21 (3)	23.7 (3.75)
f b	brown-yellow agouti and yellow	brown	25 (4)	23.7 (3.75)
e^p *a F B*	black and red	black	15 (4)	23.7 (3.75)
F b	brown and red	brown	24 (6)	23.7 (3.75)
f B	black and yellow	black	27 (3)	23.7 (3.75)
f b	brown and yellow	brown	21 (3)	23.7 (3.75)
Total			379 (30)	379.2 (30.0)

TABLE 3

The segregation of color varieties in experiment (AE)E. Genotype E A c^d p was probably not distinguished from e–c^dp in all cases. There was also segregation of genes R r, M m, and Px px in this experiment.

GENOTYPE	COAT COLOR	EYE COLOR	OBSERVED NUMBER	EXPECTED NUMBER
E A C P	black-red agouti	black	14	14.5
C p	pale sepia-red agouti	pink	10	14.5
c^d *P*	sepia-cream agouti	black	20	14.5
c^d *p*	v. p. sepia-cream agouti	pink	4	14.5
E a C P	black	black	17	14.5
C p	pale sepia	pink	17	14.5
c^d *P*	sepia	black	18	14.5
c^d *p*	very pale sepia	pink	11	14.5
e – C P	red	black	35	29.0
C p	red	pink	30	29.0
c^d *P*	cream	black	24	29.0
c^d *p*	cream	pink	32	29.0
Total			232	232.0

Table 3 shows the color varieties in experiment (AE)E. Since *Aa* and *aa* are indistinguishable in self yellows (*ee*), there are only 12 recognizable classes. The agreement with expectation is fairly good as a whole ($\chi^2 = 16.1$, $n = 11$, $P = .14$). There is, however, a rather serious deficiency in one class—*E A* c^d *p*. These are close to pink-eyed cream or yellow (*e* c^d *p*) in appearance, and it is possible that the agouti pattern was overlooked in a few

TABLE 4

The segregation according to color variety and hair direction and classification by number of digits on hind feet in experiment (AD)D. Those classified as agouti probably included some e^p *A.*

GENOTYPE	COAT COLOR	HAIR DIRECTION	NUMBERS				NO. IN COLOR VARIETIES	
			3-TOED	POOR 4-TOED	GOOD 4-TOED	TOTAL	OBS.	EXP.
E A R M	agouti	partial rough	13	15	5	33		
R m	agouti	full rough	11	7	8	26	115	89.75
r –	agouti	smooth	31	14	11	56		
e^p *A R M*	agouti and red	partial rough	12	8	2	22		
R m	agouti and red	full rough	11	8	2	21	78	89.75
r –	agouti and red	smooth	19	10	6	35		
E a R M	black	partial rough	10	12	1	23		
R m	black	full rough	9	6	7	22	85	89.75
r –	black	smooth	16	13	11	40		
e^p *a R M*	black and red	partial rough	7	8	7	22		
R m	black and red	full rough	7	8	3	18	81	89.75
r –	black and red	smooth	14	18	9	41		
Total			160	127	72	359	359	359.0

cases. The number of possible errors is too small to have appreciable effects on estimates of linkage from the whole body of data.

Tables 4, 5, and 6 give the backcross or F_2 generation in the experiments involving normal polydactyly. The frequencies of the color varieties in table 4 (experiment (AD)D) depart rather seriously from expectation ($\chi^2 = 9.74$, $n = 3$, $P = .02$). There is a marked excess of self agoutis (*E A*) and considerable defect in the class of agouti tortoiseshells (*e*^{p} *A*). The tortoiseshell pattern was often restricted to a few yellow hairs in this population, which is three-fourths blood of strain D. The agoutis in this (and the other experiments) were gone over with great care, but a few yellow hairs, conspicuous on a black ground, may have been overlooked in some cases in agoutis and would necessarily be unrecognizable if restricted to the belly.

TABLE 5

The segregation of color varieties and classification by number of digits on hind feet in experiment (CD)². There were nine young which could not be classified as F or f because of absence of red or yellow hairs.

GENOTYPE	COAT COLOR	EYE COLOR	NUMBERS			TOTAL	
			3-TOED	POOR 4-TOED	GOOD 4-TOED	OBS.	EXP.
C F P	black and red	black	76	13	9	98	121.1
C – P	black	black	6	3	0	9	—
C f P	black and yellow	black	30	9	2	41	40.4
c^d F P	sepia and yellow	black	26	10	0	36	40.4
c^d f P	sepia and pale cream	black	8	5	0	13	13.4
C F p	pale sepia and red	pink	37	16	5	58	40.4
C f p	pale cream and yellow	pink	10	6	2	18	13.4
c^d F p	v.p. sepia and yellow	pink	6	5	0	11	13.4
c^d f p	white and pale cream	pink	2	1	0	3	4.5
Total			201	68	18	287	287.0

In experiment (CD)² (table 5) there were no difficulties of classification except that nine blacks could not be classified as *Ff* or *ff* because of absence of red or yellow hairs. These animals illustrate the occasional overlap of $e^v e^v$ with Ee^v even in blacks, since all of them were $e^v e^v$. Grouping together the blacks, black-reds, and black-yellows, the agreement with ex-

TABLE 6

The segregation of coat colors and classification by number of digits on the hind feet in experiment (BD)². F_1 was s c^k p b 3-toed × S C P B 4-toed.

GENOTYPE	COAT COLOR	EYE COLOR	S LITTLE OR NO WHITE			s WHITE SPOTTED			TOTAL	
			3-TOED	POOR 4-TOED	GOOD 4-TOED	3-TOED	POOR 4-TOED	GOOD 4-TOED	OBS.	EXP.
C P B	black and red	black	104	29	4	25	11	3	176	162.0
P b	brown and red	brown	29	10	0	7	6	1	53	54.0
p B	p. sepia and red	pink	27	5	2	11	2	0	47	54.0
p b	p. brown and red	pink	6	4	0	1	1	0	12	18.0
c^k P B	sepia and yellow	black	28	7	3	10	2	2	52	54.0
P b	brown and yellow	brown	8	2	1	1	0	0	12	18.0
p B	p. sepia and yellow	pink	14	3	0	5	0	1	23	18.0
p b	p. brown and yellow	pink	3	2	0	3	1	0	9	6.0
Total			219	62	10	63	23	7	384	384.0

pectation is only moderately good ($\chi^2 = 11.80$, n = 6, P = .07). The largest contribution to χ^2, however, is in the class of pink-eyed pale sepia tortoiseshells which could not have been confused with anything else.

In experiment $(BD)^2$ (table 6) there were no difficulties of classification (apart from that of *Ss* and *ss* already discussed). The eight color varieties, disregarding white spotting, agree well with expectation ($\chi^2 = 9.10$, n = 7, P = .25).

R is the gene usually responsible for the occurrence of rosettes in the coat of guinea pigs. There is, however, another dominant gene *Wl* which causes a well marked whorl on the forehead (tracing wholly in my stock to three animals kindly presented by MR. I. J. WACHTEL). This has been shown to be independent of *R*. Other strains show irregularities of hair direction on the belly and occasionally on the back, without either *R* or *Wl*. The most reliable indicator of *R* is reversal of hair direction on the hind toes.

The most important variations in the number of rosettes in animals with *R* are due to genes *Mm*. These produce no effect in smooth furred animals. *R mm* is usually "full rough" with well developed rosettes on head, at least two pairs on the back, irregularities on the belly, and reversal on the feet. *R Mm* is usually without rosettes on head or belly. There is only one pair on the back, often reduced to a mere crest. *R MM* is usually smooth furred except for the reversal on the feet and occasionally cannot be distinguished from smooth. There was no possibility, however, of confusing *R* and *rr* in the main experiment, except in a very few cases of matings that proved to be *Rr Mm*×*rr Mm* instead of *Rr Mm*×*rr mm* and which thus produced *R MM*.

In one experiment, which is not included here, a modifier or modifiers were present which increased the number of rosettes in *R Mm* to such an extent that there was overlap with *R mm* (WRIGHT 1935b). There is no indication that this occurred in the experiments considered here. Modifiers were sometimes present in (AF)F, however, which reduced *R mm* to a type with only one pair of dorsal rosettes, but still with head rosettes and irregularities on the belly. From the results of breeding tests it appears that *R mm* and *R Mm* could be distinguished with few if any mistakes in these experiments.

The expression of *Px* is highly variable in heterozygotes and fails altogether in a considerable percentage of cases (WRIGHT 1835a). The homozygotes are monstrous and inviable. The most reliable indicator of *Px px* is the presence of the pollex, which has been described only twice in *px px*. One of these exceptions was an animal of strain F, *px px* by ancestry and a thorough breeding test. The other was a hybrid between the guinea pig and *Cavia rufescens* described by DETLEFSEN (1914). The hallux is an equally

good indicator when present but is much less regularly present in *Px px* than is the thumb. One or both of the little toes are usually present in *Px px* but are less reliable indicators than the thumb or big toe since they also often occur in *px px*. Thus CASTLE developed by selection a strain (D of the present paper) which invariably had well developed little toes, but never thumbs or big toes (CASTLE 1906). This normal polydactyly is due to multiple factors (WRIGHT 1934). These have been shown to accentuate the development of thumbs and big toes, as well as little toes in *Px px* (WRIGHT 1935a). Strain F by itself has produced 815 normal, 170 with one or both little toes weakly developed (including one with a thumb, referred to above), and 21 with both little toes well developed. Thus it has more plus modifiers for polydactyly than the ordinary normal strains. In the portion of (AF)F made before introduction of *Px*, there were seven in 188 or 3.7 percent with little toes. Where the AF parent was *Px px* there were 39.6 percent (of 230) with pollex, 13.0 percent with little toes but no pollex, and 47.4 percent normal. It appears from these ratios that most of the second class was *Px px* but probably not all. For purposes of testing linkage, it has seemed best to treat these as all *Px*, *px*.

Strain E was highly heterogeneous but almost invariably normal with respect to digits. In the backcross generation (AE)E the ratio of 78 with at least one thumb:nine with little toes but no thumb:141 normal indicates that all of the small second class can safely be treated as *Px px* in this case. Including these the ratio is 87 *Px px*:141 apparent *px px* with a deviation of 3.6 times the standard error from the expected one:one ratio. There is no doubt that a considerable number with only four toes on the front feet and three on the hind feet were really *Px px*. On tabulating the results of matings separately it was found that in three cases the probability of the deviation from expected equality was about .01 or less (1:13, 1:10, 0:8) while in no other cases was the probability less than .05 (extreme cases 1:6, 0:4). Some matings with moderately large numbers gave no indication of any deficiency of *Px px* (10:8, 8:5, 9:7, 8:7, 11:13 etc.). It appears that the above three matings with a total ratio of 2:31 could be of little value in tests for linkage. Omitting them, the ratio of the remainder, 85:110, does not differ seriously from 1:1.

The sex ratios and the one factor ratios are given by experiment in table 7. The only deviations from expectation that exceed twice the standard error are in connection with *Px*, *px*, discussed above; *E*, e^p; and in one experiment *P*, *p*. The apparent ratio of Ee^p to e^pe^p in (AB)B, (AD)D, and (AF)F combined was 667:570, a deviation from expectation of 2.76 times its standard error. There can be little doubt that about 8 percent of e^pe^p were classified as Ee^p because of overlap.

The aberrant ratio of 197 *P* to 90 *pp* in $(CD)^2$, where a 3:1 ratio is ex-

TABLE 7

The percentages of recessives (males in case of sex) in the various experiments. The wide departure from expectation for Px:px in (AE)E is largely due to three matings which gave only two polydactyls in 33 young. Excluding these the percentage of normals was 56.4, and Dev./S.E. was only 1.8. These matings were excluded in other tests involving Px.

ALLELES	EXPERIMENT	NO.	PERCENT RECESSIVE	DEV./S.E.	ALLELES	EXPERIMENT	NO.	PERCENT RECESSIVE	DEV./S.E.
♀:♂	(AB)B	486	47.9	0.9	*F:f*	(AF)F	329	46.5	0.6
"	(AD)D	359	45.7	1.6	"	(CD)[2]	278	27.0	0.8
"	(AF)F	417	48.9	0.4					
"	(AE)E	230	55.7	1.7	*P:p*	(AB)B	461	50.3	0.1
"	(BD)[2]	380	47.9	0.8	"	(AE)E	232	44.8	1.6
"	(CD)[2]	285	46.7	1.1	"	(BD)[2]	384	23.7	0.6
					"	(CD)[2]	287	31.4	2.5
S:s	(AB)B	460	49.4	0.3					
"	(AF)F	285	46.7	1.1	*B:b*	(AB)B	458	47.6	1.0
"	(BD)[2]	384	24.2	0.4	"	(AF)F	423	52.3	0.9
					"	(BD)[2]	384	22.4	1.2
E:e[p]	(AB)B	489	46.8	1.4					
"	(AD)D	359	44.3	2.2	*R:r*	(AB)B	471	50.1	0.1
"	(AF)F	389	46.8	1.3	"	(AD)D	357	48.2	0.7
E:e	(AE)E	233	52.4	0.7	"	(AF)F	420	50.7	0.3
					"	(AE)E	232	52.2	0.7
A:a	(AB)B	454	50.2	0.1					
"	(AD)D	359	46.2	1.4	*M:m*	(AB)B	247*	50.6	0.2
"	(AF)F	421	47.0	1.2	"	(AD)D	187*	46.5	1.0
"	(AE)E	111	56.8	1.4	"	(AF)F	205*	47.8	0.6
					"	(AE)E	97*	45.4	0.9
$C:c^k$	(AB)B	363	47.1	1.1					
$C:(c^k)c^dc^r$	(AE)E	231	46.8	1.0	*Px:px*	(AF)F	230	47.4	0.8
$C:c^k$	(BD)[2]	384	25.0	0.0	"	(AE)E	228	61.8	3.6
$C:c^d$	(CD)[2]	287	22.0	1.2					

* In *R* class only.

pected, deviates by 2.5 times its standard error. There is no possibility of confusion here, and in the two other experiments showing segregation of these genes (including one in which *P* came from the same stock at the same time), the deviation is in the other direction (to insignificant extents). No interpretation is apparent other than an extreme chance deviation.

TESTS FOR PARTIAL SEX LINKAGE

From cytological observations it appears that the male is heterogametic in guinea pigs as in other mammals, but there is disagreement on whether a Y chromosome is present or not, as well as serious disagreement on the total number of chromosomes, estimates running from 16 to 65 (in males). The most recent estimates are those of LEAGUE (1928), who found about 31

TABLE 8

Tests for occurrence of partial sex linkage on hypothesis that males are heterogametic. In progeny of female recessive by male heterozygotes from female recessive by male dominant (R(RD)), the female dominants and the male recessives are the recombination classes. The situation is reversed in R(DR).

LOCUS	R(RD) DOMINANT ♂	R(RD) DOMINANT ♀	R(RD) RECESSIVE ♂	R(RD) RECESSIVE ♀	R(RD) TOTAL	R(RD) % RECOMB.	R(RD) DEV. S.E.	R(DR) DOMINANT ♂	R(DR) DOMINANT ♀	R(DR) RECESSIVE ♂	R(DR) RECESSIVE ♀	R(DR) TOTAL	R(DR) % RECOMB.	R(DR) DEV. S.E.
S	19	18	8	23	68	38.2	1.9	71	74	59	67	271	50.9	0.3
E	83	67	83	78	311	48.2	0.6	132	160	105	125	522	49.2	0.4
A	64	51	57	67	239	45.2	1.5	117	149	121	133	520	48.1	0.9
C	53	41	53	34	181	51.9	0.5	37	48	30	40	155	49.7	0.1
F	20	23	23	28	94	48.9	0.2	27	25	14	21	87	55.2	1.0
P	56	41	59	42	198	50.5	0.1	50	63	49	54	216	48.2	0.5
B	34	49	47	47	177	54.2	1.1	72	91	73	78	314	47.8	0.8
R	87	77	84	75	323	49.9	0.0	118	150	115	129	512	48.2	0.8
M	46	34	39	42	161	45.3	1.2	64	79	54	71	268	50.4	0.2
Px	52	53	71	51	227	54.6	1.4	9	12	8	18	47	57.5	1.0

pairs including a possible XY pair, and of MOLS (1928), who was confident that there are just 32 pairs of autosomes and an unpaired X which goes to one pole of the primary spermatocyte. Partial sex linkage is possible if there is an XY pair but not if there is an unpaired X.

The backcrosses have been made in four ways with respect to sex. Writing females first in this section and letting D stand here for dominant, R for recessive, these may be represented R(RD), R(DR), (RD)R, and (DR)R, respectively. If there is an XY pair in males, the female dominants and male recessives are the recombination classes in R(RD) for partially sex linked genes. In R(DR) the female recessives, and the male dominants are the recombination classes. Crosses (RD)R and (DR)R give no information on this question unless it is the females that are heterogametic, in which case the female dominants and male recessives are the recombination classes in (RD)R, while the opposite associations constitute the recombination classes in (DR)R. Whichever sex is heterogametic, $(RD)^2$ gives $(\frac{1}{4})$ (2-p) male dominants, p/4 male recessives, $(\frac{1}{4})$ (1+p) female dominants, and $(\frac{1}{4})$ (1−p) female recessives, where p is the amount of recombination in the heterogametic sex. In $(DR)^2$ these frequencies hold with reversal of sex.

The data are shown in table 8, 9, 10, and 11. The standard errors of the recombination percentages from the backcross data are based on 50 percent recombination, expected if the gene is autosomal. In the case of the F_2 data, the few data from $(DR)^2$ were combined with $(RD)^2$, with reversal of sex for estimation of the recombination percentage which would yield the ob-

TABLE 9

Tests for occurrence of partial sex linkage on hypothesis that females are heterogametic. In progeny of (RD)R female dominants and male recessives are the recombination classes. The situation is reversed in (DR)R.

LOCUS	(RD)R DOMINANT ♂	(RD)R DOMINANT ♀	(RD)R RECESSIVE ♂	(RD)R RECESSIVE ♀	(RD)R TOTAL	(RD)R % RECOMB.	(RD)R DEV. S.E.	(DR)R DOMINANT ♂	(DR)R DOMINANT ♀	(DR)R RECESSIVE ♂	(DR)R RECESSIVE ♀	(DR)R TOTAL	(DR)R % RECOMB.	(DR)R DEV. S.E.
S	44	31	40	36	151	47.0	0.7	68	55	51	73	247	57.1	2.2
E	69	46	48	54	217	43.3	1.9	107	108	84	105	404	52.5	1.0
A	56	45	49	42	192	49.0	0.3	91	110	86	93	380	48.4	0.6
C	34	29	30	35	128	46.1	0.9	35	33	21	35	124	56.5	1.4
F	15	9	12	17	53	39.6	1.5	30	23	15	22	90	57.8	1.5
P	46	32	33	37	148	43.9	1.5	29	36	25	37	127	52.0	0.4
B	41	35	48	33	157	52.9	0.7	53	58	52	57	220	50.0	0.0
R	62	47	53	52	214	46.7	1.0	95	98	103	125	421	52.3	0.9
M	34	21	22	25	102	42.2	1.6	46	47	48	46	187	49.2	0.2
Px	26	21	24	15	86	52.3	0.4	18	15	12	18	63	57.1	1.1

served figures with maximum likelihood. The general equation to be solved is as follows where f_0 is the observed and f_e the calculated frequency of a class (FISHER 1938):

$$\Sigma\left[f_0 \frac{d \log f_e}{dp}\right] = 0.$$

This takes the following form where (D♂) etc., are the observed frequencies:

$$-\frac{(D♂)}{2-p} + \frac{(R♂)}{p} + \frac{(D♀)}{1+p} - \frac{(R♀)}{1-p} = 0$$

TABLE 10

Sex ratios of dominants and recessives in F_2. $(DR)^2$ combined with $(RD)^2$ with reversal of sex in calculation of recombination percentage.

LOCUS	$(RD)^2$ DOMINANT ♂	$(RD)^2$ DOMINANT ♀	$(RD)^2$ RECESSIVE ♂	$(RD)^2$ RECESSIVE ♀	$(RD)^2$ TOTAL	$(RD)^2$ % RECOMB.	$(RD)^2$ DEV. S.E.	$(DR)^2$ DOMINANT ♂	$(DR)^2$ DOMINANT ♀	$(DR)^2$ RECESSIVE ♂	$(DR)^2$ RECESSIVE ♀	$(DR)^2$ TOTAL
S	143	146	39	52	380	45.1	1.1	—	—	—	—	—
C	240	248	66	86	640	44.7	1.6	6	15	3	1	25
F	88	96	29	38	251	44.8	1.0	5	13	4	3	25
P	227	242	79	92	640	49.2	0.2	7	9	2	7	25
B	144	151	38	47	380	47.2	0.6	—	—	—	—	—

The standard error derived from the general formula (FISHER 1938),

$$(1/\sigma_p^2) = -n\Sigma\left[f_c \frac{d^2 \log f_c}{dp^2}\right]$$

on the hypothesis that p is expected to be .50, is $\sqrt{3/4n}$.

There is only one case in the tables in which the observed frequency exceeds twice its standard error. This is in the case of *S* in experiment (DR)R

TABLE 11

Estimates of percentages of recombination with sex based on combination of backcross and F_2 data as described in text.

LOCUS	TESTS OF MALES		TESTS OF FEMALES	
	% RECOMB.	DEV. / S.E.	% RECOMB.	DEV. / S.E.
S	47.5	1.1	51.3	0.6
E	48.9	0.7	49.3	0.4
A	47.2	1.6	48.6	0.7
C	48.4	0.7	48.2	0.8
F	49.5	0.2	48.6	0.4
P	49.3	0.4	48.4	0.7
B	49.5	0.3	50.2	0.1
R	48.9	0.7	50.4	0.2
M	48.5	0.6	46.7	1.1
Px	55.1	1.7	54.4	1.1

where the recombination percentage (57.1 percent) is 2.23 times its standard error. This would indicate partial sex linkage only if the female is the heterogametic sex and only if the conditions of crossing over are such that recombination may exceed 50 percent. It is most probable that this is merely an extreme chance deviation. The tests of all heterozygous males and those of all heterozygous females are shown in table 11. In combining the back cross and F_2 data, the estimates were weighted by the inverse squares of their standard errors and the averages were assigned standard errors from the square roots of the reciprocal of the sum of these weights. There is no indication of partial sex linkage that need be taken seriously.

TESTS FOR LINKAGE AMONG WELL DEFINED GENES

Table 12 shows the backcross data for each combination of two series of alleles. The tests of F_1 males and F_1 females are given separately. The different experiments are combined except in certain cases in which different alleles were used; in cases in which complete classification could not be

TABLE 12

Backcross tests for linkage. All of the data is from coupling experiments except in the case of R px/r Px. UV is the class of double dominants, Uv that in which the first series under "F_1 parent" is represented by the dominant, the second by the recessive, uV *the reverse and* uv *the class of double recessives. Compound superscripts are used in tables 12 and 13 to represent alternative alleles at the C locus. In certain cases indicated by such expressions as "in E A" and "excl e" (excluding e) certain segregating genotypes could not be distinguished.*

F_1 PARENT	TESTS OF F_1 FEMALES							TESTS OF F_1 MALES						
	UV	Uv	uV	uv	TOTAL	% RE-COMB.	DEV. / S.E.	UV	Uv	uV	uv	TOTAL	% RE-COMB.	DEV. / S.E.
S E/s e^p	106	88	100	88	382	49.2	0.3	89	84	84	69	326	51.5	0.6
S A/s a	95	94	98	84	371	51.8	0.7	88	92	76	79	335	50.2	0.1
S C/s c^k	45	35	45	44	169	47.3	0.7	49	38	37	40	164	45.7	1.1
S F/s f (in *E A*)	13	8	14	10	45	48.9	0.2	10	6	5	8	29	37.9	1.3
S F/s f (in e^p)	26	24	21	22	93	48.4	0.3	14	17	15	12	58	55.2	0.8
S P/s p	48	45	48	52	193	48.2	0.5	68	58	54	58	238	47.1	0.9
S B/s b	93	95	92	92	372	50.3	0.1	91	89	81	78	339	50.2	0.1
S R/s r	91	109	97	102	399	51.6	0.7	80	92	83	69	324	54.0	1.4
S M/s m	46	45	57	40	188	54.3	1.2	39	47	43	46	175	51.4	0.4
S Px/s px	27	16	18	12	73	46.6	0.6	4	6	9	3	22	68.2	1.7
E A/e^pa	151	123	123	113	510	48.2	0.8	199	183	149	154	685	48.5	0.8
E A/e a (excl. *e*)	22	25	—	—	47	53.2	0.4	26	38	—	—	64	59.4	1.5
E C/e^p c^k (excl. *E a*)	37	32	53	47	169	50.3	0.1	32	30	67	61	190	51.1	0.3
E C/e c^{dr}	23	24	19	18	84	51.2	0.2	35	29	46	35	145	51.7	0.4
E F/e^p f(in *E A*)	28	18	—	—	46	39.1	1.5	42	26	—	—	68	38.2	1.9
E F/e^p f (in e^p)	—	—	39	42	81	48.2	0.3	—	—	47	54	101	46.5	0.7
E P/e^p p	49	61	47	36	193	56.0	1.7	66	73	66	62	267	52.1	0.7
E P/e p	31	16	17	20	84	39.3	2.0	38	26	42	39	145	46.9	0.8
E B/e^p b	98	100	84	80	362	50.8	0.3	126	132	114	108	480	51.3	0.6
E R/e^p r	140	142	119	134	535	48.8	0.6	198	177	150	152	677	48.3	0.9
E R/e r	19	28	17	20	84	53.6	0.7	36	28	39	42	145	46.2	0.9
E M/e^p m	76	64	57	60	257	47.1	0.9	104	99	77	80	360	48.9	0.4
E M/e m	6	7	5	7	25	48.0	0.2	22	13	20	17	72	45.8	0.7
E Px/e^p px	22	12	20	10	64	50.0	0.0	36	36	27	35	134	47.0	0.7
E Px/e px	20	21	11	18	70	45.7	0.7	23	30	29	40	122	48.4	0.4
A C/a c^k (excl. *E a*)	56	50	19	17	142	48.6	0.3	57	61	37	25	180	54.4	1.2
A C/a c^{dr}	11	11	12	13	47	48.9	0.2	13	13	22	16	64	54.7	0.8
A F/a f (in *E A*)	28	18	—	—	46	39.1	1.5	42	26	—	—	68	38.2	1.9
A F/a f (in e^p	28	19	22	27	96	42.7	1.4	27	35	28	27	117	53.8	0.8
A P/a p	70	58	56	56	240	47.5	0.8	76	68	90	88	322	49.1	0.3
A B/a b	96	102	92	87	377	51.5	0.6	132	115	116	130	493	46.9	1.4
A R/a r	141	159	135	135	570	51.6	0.8	198	176	194	181	749	49.4	0.3
A M/a m	69	69	69	61	268	51.5	0.5	108	97	103	95	403	49.6	0.2
A Px/a px	35	25	34	26	120	49.2	0.2	52	48	43	60	203	44.8	1.5
C P/c^{kdr} p	56	62	67	42	227	56.8	2.1	98	82	77	81	338	47.0	1.1
C B/c^k b	40	35	30	37	142	45.8	1.0	55	42	48	38	183	49.2	0.2
C R/c^{kd} r	62	69	53	68	252	48.4	0.5	94	78	70	80	322	46.0	1.5
C M/c^{kdr} m	29	24	21	30	104	43.3	1.4	48	49	40	34	171	52.1	0.5
C Px/c^{dr} px	18	18	13	21	70	44.3	1.0	29	38	24	33	124	50.0	0.0
F B/f b (in *E A*)	12	16	10	8	46	56.5	0.9	15	27	16	10	68	63.2	2.2
F B/f b in e^p)	24	26	26	21	97	53.6	0.7	26	30	28	34	118	49.2	0.2
F R/f r (in *E A*)	12	15	7	11	45	48.9	0.2	23	19	15	11	68	50.0	0.0
F R/f r in e^p)	23	26	18	29	96	45.8	0.8	23	33	38	24	118	60.2	2.2
F M/f m in *E A*)	7	5	5	2	19	52.6	0.2	9	14	11	4	38	65.8	2.0
F M/f m (in e^p)	12	9	11	7	39	51.3	0.2	8	15	19	19	61	55.7	0.9
F Px/f px in *E A*)	7	4	5	3	19	47.4	0.2	13	14	6	7	40	50.0	0.0
F Px/f px (in e^p	14	11	12	8	45	51.1	0.2	20	15	16	27	78	39.7	1.8
P B/p b	45	51	48	49	193	51.3	0.4	72	60	71	58	261	50.2	0.1
P R/p r	67	77	67	65	276	52.2	0.7	104	100	97	97	398	49.5	0.2
P M/p m	31	30	29	33	123	48.0	0.5	55	52	53	50	210	50.0	0.0

TABLE 12 (*Continued*)

F_1 PARENT	TESTS OF F_1 FEMALES							TESTS OF F_1 MALES						
	UV	Uv	uV	uv	TOTAL	% RECOMB.	DEV./S.E.	UV	Uv	uV	uv	TOTAL	% RECOMB.	DEV./S.E.
P Px/p px	20	23	11	16	70	48.6	0.2	22	41	32	30	125	58.4	1.9
B R/b r	88	98	93	96	375	50.9	0.4	130	111	118	121	480	47.7	1.0
B M/b m	46	41	52	40	179	52.0	0.5	68	67	56	69	260	47.3	0.9
B Px/b px	24	13	25	17	79	48.1	0.3	31	39	41	40	151	53.0	0.7
R M/r m (excl *r*)	149	141	—	—	290	48.6	0.5	225	207	—	—	432	47.9	0.9
R Px/r px	43	25	35	43	146	41.1	2.2	76	68	50	82	276	42.8	2.4
R px/r Px	5	9	8	6	28	39.3	1.1	8	25	21	26	80	42.5	1.3
M Px/m px	19	12	18	8	57	52.6	0.4	41	34	35	32	142	48.6	0.3

made, giving an expected ratio other than 1:1:1:1, and in all cases involving *F*, *f* in which, as discussed above, classification is more reliable in tortoiseshells (e^p) than in self agouti (*E A*). The data from (AB)B of a previous paper are included (WRIGHT 1928).

Table 13 shows the F_2 data. It includes not only $(BD)^2$ and $(CD)^2$, discussed in a previous section, but data on the relation of genes *C*, *F*, and *P*

TABLE 13

F_2 and allied tests for linkage.

F_1 GENOTYPES	UV	Uv	uV	uv	TOTAL	% RECOMB.	DEV./S.E.
$(S\ C/s\ c^k)^2$	220	71	68	25	384	48.2	0.5
$(S\ P/s\ p)^2$	225	66	68	25	384	46.8	0.8
$(S\ B/s\ b)^2$	226	65	72	21	384	49.7	0.1
$C\ F/c^d\ f)^2$	156	59	47	16	278	51.7	0.4
$(C\ f/c^{dr}\ F)^2$	93	32	28	11	164	51.8	0.3
$(C\ P/c^{dr}\ p)^2$	473	164	142	58	837	47.8	0.9
$(C\ P/c^k\ p)^2$	223	65	75	21	384	50.6	0.2
$(F\ P/f\ p)^2$	134	69	54	21	278	53.4	0.8
$(F\ p/f\ P)^2$	130	45	53	12	240	43.9	1.3
$(P\ B/p\ b)^2$	228	65	70	21	384	49.1	0.2
$C\ f/c^d\ F \times c^d\ f/c^d\ F$	25	13	29	9	76	40.5	1.0
$C\ P/c^d\ p \times c^d\ P/c^d\ p$	29	9	31	7	76	55.9	0.6

published previously (WRIGHT 1927). The recombination percentages in the F_2 data were estimated by the method of maximum likelihood. The equation to be solved takes the form

$$\frac{UV}{2+x} - \frac{(Uv + uV)}{1-x} + \frac{(uv)}{x} = 0$$

where $x = (1\text{-}p_1)\ (1\text{-}p_2)$ in coupling data, $x = p_1p_2$ in repulsion data, p_1 and p_2 being the amounts of recombination in the two sexes. The standard error is $3/(4\sqrt{n})$ on the hypothesis of independence ($p_1 = p_2 = \frac{1}{2}$).

Table 14 gives the final estimate of recombination percentages and the standard errors on the hypothesis of independence, based on both back cross and F_2 data as in the discussion of partial sex linkage.

TABLE 14

Tests for linkage. Estimates of recombination percentage from backcross, F_2 and miscellaneous crosses. Tests for linkage of EF and AF exclude EA (38.6 percent apparent recombination of either E or A with F Dev./S.E.=2.4) because of the uncertainty of classification of F in this combination.

LOCI	% RECOMB.	DEV./S.E.	LOCI	% RECOMB.	DEV./S.E.	LOCI	% RECOMB.	DEV./S.E.
S E	50.3	0.2	*E M*	47.9	1.1	*F P*	49.0	0.3
S A	51.0	0.5	*E Px*	47.7	0.9	*F B*	54.4	1.6
S C	47.1	1.3	*A C*	52.0	0.8	*F R*	52.3	0.8
S F	48.9	0.3	*A F*	48.8	0.3	*F M*	56.7	1.7
S P	47.3	1.3	*A P*	48.4	0.8	*F Px*	45.6	1.2
S B	50.1	0.1	*A B*	48.9	0.7	*P B*	50.2	0.1
S R	52.7	1.5	*A R*	50.3	0.3	*P R*	50.6	0.3
S M	52.9	1.1	*A M*	50.4	0.2	*P M*	49.3	0.3
S Px	51.6	0.3	*A Px*	46.4	1.3	*P Px*	54.9	1.4
E A	49.1	0.7	*C F*	50.1	0.0	*B R*	49.1	0.5
E C	51.0	0.5	*C P*	49.9	0.1	*B M*	49.2	0.3
E F	47.3	0.7	*C B*	48.7	0.6	*B Px*	51.3	0.4
E P	50.5	0.3	*C R*	47.0	1.4	*R M*	48.2	1.0
E B	51.1	0.6	*C M*	48.7	0.4	*R Px*	42.1	3.7
E R	48.6	1.1	*C Px*	47.9	0.6	*M Px*	49.8	0.1

Inspection of these tables reveals only one case in which linkage is strongly indicated. This is in the tests of both males and females of constitution *R Px/r px*. These agree closely in the amount of recombination: males 42.75 ± 3.01 percent, females 41.10 ± 4.14 percent. After finding this result, a search was made through the records for other matings which would bear on this question. In a considerable number of cases crosses were made between the old strain A (of constitution *R px/R px*) and smooth furred polydactyls *r Px/r px*. Some of the progeny were used as multiple dominants in F_1 of AE and AF in matings of type *R px/r Px* × *r px/r px* and thus gave repulsion data. In a considerable number of these the rough modifier *M* inadvertently came in from both parents, resulting in *R MM* offspring which may have been confused with smooth young (*rr*) in some cases. The penetrance of *Px* was also rather low in AE. The ratio in the case of tests of males is thus rather aberrant, apart from indications of linkage. Nevertheless, the amount of linkage indicated by these repulsion data is in good agreement with that from the coupling data. The total from all data, 42.08 ± 2.17, deviates from 50 percent by 3.65 times the standard error and thus would be equalled or exceeded by accidents of sampling only two or three times in 10,000.

The next strongest indications of linkage are for *E* and *F* (43.9 ± 2.9 percent, deviation 2.1 times standard error) and *A* and *F* (45.3 ± 2.8 percent deviation 1.7 times standard error). Part of the data on which these are based involves classification of doubtful accuracy. As already pointed out, *Ff*, and *ff* cannot be distinguished at all in *EF* and *Ef* and are distinguished with some difficulty in self agoutis, *E A F* and *E A f*. The ratio 70 *E A F* to 44 *E A f* might indicate linkage of *F* to either *E* or *A* but is more probably due to errors in classification. If only tortoiseshells are considered the recombination percentage for *E* and *F* becomes 47.3 ± 3.7, deviation 0.7 times standard error and that for *A* and *F* becomes 48.8 ± 3.4, deviation 0.3 times standard error. In neither case is there serious evidence of linkage.

There are four other cases in which the deviation from 50 percent equals or exceeds twice the standard error in portions of the data for crossbreds of one sex, but none of these is borne out by the rest of the data from the same sex or from the other sex.

The most probable interpretation at present is that the genes studied are all independent of each other and of sex with the exception of *R* and *Px*.

LINKAGE TESTS INVOLVING NORMAL POLYDACTYLY

Experiments (AD)D, $(BD)^2$, and $(CD)^2$ and reciprocals were made in the hope of throwing light on the genetics of normal polydactyly. As noted earlier, strain D was characterized by regular development of the little toes and thus exhibited four toes on the hind feet instead of the usual three. Matings were made with the regularly 3-toed strains A and B. Strain D became extinct in the course of the experiments because of the extremely low fertility of the females, but good 4-toed animals that were seven-eighths blood or more of strain D were available and were used to a greater extent than pure D in backcrossing to AD and in starting CD.

Data with respect to normal polydactyly are presented in table 15. It may be seen that all of the F_1's are closely similar: about 90 percent 3-toed and the rest weakly 4-toed except for one good 4-toed individual in CD. The F_2 data are classified both by experiment and by the character of the F_1 parents: 3-toed × 3-toed, 3-toed × 4-toed (and reciprocal), and 4-toed × 4-toed. The character of the parents made no significant difference in $(BD)^2$ (grouping all 4-toed together, $\chi^2 = 1.4$, n = 2, P = about .50). In this case pure D and a somewhat closely inbred strain of 3-toed animals were used. There is a highly significant difference in relation to the character of the F_1 parents in $(CD)^2$ in which both parental strains were less homogeneous ($\chi^2 = 40.9$, $n = 2$, probability negligible that differences are accidental.)

The data given here are compatible with segregation of a single pair of alleles with the 3-toed condition usually dominant (90 percent 3-toed in F_1, 72 percent 3-toed in F_2, 45 percent 3-toed in the backcross). But a

TABLE 15

Distribution with respect to normal polydactyly in pure strains, F_1, backcrosses and F_2.

GENERATION		EXPERIMENT	PERCENTAGE			
			3-TOED	POOR 4-TOED	GOOD 4-TOED	TOTAL NUMBER
Pure strains		D	—	—	100.0	49
		A	100.0	—	—	360
		B	100.0	—	—	907
		C	100.0	—	—	380
F_1		AD	90.2	9.8	—	82
		BD	91.2	8.8	—	68
		CD	89.7	9.0	1.3	78
		DC	94.4	5.6	—	18
		Total	90.3	9.3	0.4	246
Backcross		D(AD)	46.9	34.4	18.7	209
		(AD)D	41.3	36.7	22.0	150
		Total	44.6	35.4	20.0	359
F_2	3T×3T	$(BD)^2$	74.0	22.3	3.7	296
		$(DC)^2$	76.0	20.0	4.0	25
		$(CD)^2$	83.7	14.4	2.0	153
	3T×4T	$(BD)^2$	73.7	18.4	7.9	76
		$(CD)^2$	59.6	28.1	12.3	57
	4T×4T	$(BD)^2$	58.3	41.7	—	12
		$(CD)^2$	38.5	48.1	13.5	52
		Total	72.0	22.8	5.2	671

TABLE 16

Distribution with respect to normal polydactyly and sex. The column Dev./S.E. gives the ratio of the difference in percentage of total 4-toed to its standard error. L indicates deviation in direction expected from linkage, N the reverse. (AD)D would reveal partial sex linkage only if female is heterogametic.

EXPERIMENT	MALE				FEMALE				DEV./S.E.
	PERCENTAGE			TOTAL NO.	PERCENTAGE			TOTAL NO.	
	3-TOED	POOR 4-TOED	GOOD 4-TOED		3-TOED	POOR 4-TOED	GOOD 4-TOED		
D(AD)	52.6	32.6	14.7	95	42.1	36.0	21.9	114	1.5 N
(AD)D	47.8	33.3	18.8	69	35.8	39.5	24.7	81	1.5 N
$(CD)^2$	71.8	22.6	5.6	124	68.4	25.0	6.6	136	0.6 N
$(DC)^2$	66.7	33.3	0.0	9	81.2	12.5	6.3	16	— N
$(BD)^2$	71.4	24.2	4.4	182	75.8	19.7	4.5	198	1.0 L

breeding test of the apparent segregants following crosses of strain D with other inbred strains has demonstrated that multiple genetic (and non-genetic) factors are involved (WRIGHT 1934). The amount of segregation in the most closely similar cases was such as would be found with three or four genes of equal effect.

Table 16 shows the relations of normal polydactyly to sex in the principal data. Association with sex may be tested by calculating χ^2 either from the 3×2 table (two degrees of freedom) or, less ambiguously, from the 2×2 table made by combining all polydactyls in one class. The value of χ

TABLE 17

Tests for linkage of normal polydactyly with various genes in backcrosses. Experiments D(AD) and (AD)D. Polydactyly entered the cross with the recessive alleles, L and N as in Table 15.

ALLELES	DOMINANT				RECESSIVE				DEV./S.E.
	PERCENTAGE			TOTAL NO.	PERCENTAGE			TOTAL NO.	
	3-TOED	POOR 4-TOED	GOOD 4-TOED		3-TOED	POOR 4-TOED	GOOD 4-TOED		
A a	50.3	32.1	17.6	193	38.0	39.2	22.9	166	2.3 L
E e^p	45.0	33.5	21.5	200	44.0	37.7	18.2	159	0.2 L
R r	42.8	38.5	18.7	187	46.5	32.0	21.5	172	0.7 N
M m	42.0	43.0	15.0	100	43.7	33.3	23.0	87	0.2 N

in this case is the same as the ratio of the difference to its standard error (Dev./S. E.). There is no indication of association by either calculation in any of the experiments. It appears that there is no important gene affecting normal polydactyly that is sex linked in any sense.

Table 17 shows the relation of normal polydactyly to genes *A*, *E*, *R*, and *M* in experiment (AD)D and reciprocal. There is rather strong association with *A* (P = .07 from the 3×2 table, and .02 from the 2×2 table). The deviations are such as would be expected from linkage. If polydactyly were due to a single gene, the data would indicate a recombination percentage of 44.3 ± 2.6 percent. It is thus probable that one of the major genes shows linkage with *A*. There is no indication of linkage with *E*, *R*, or *M*.

Table 18 shows F_2 from $(CD)^2$, combined with $(DC)^2$, and $(BD)^2$. These are all repulsion data, since the predominantly recessive polydactyl condition entered the crosses in association with the dominant alleles. In the case of *C*, c^d in $(CD)^2$, the probability of χ^2 from the 3×2 table is low (.02) but is due to irregularities within the 4-toed class. There is no indication of linkage on combining all polydactyls in one class. It is possible that this indicates linkage with *C* of a gene necessary for perfect development of the little toe, but if so, it was not involved in experiment $(BD)^2$. There is a

TABLE 18

Tests for linkage of normal polydactyly with various genes in F_2. $(CD)^2$ includes $(DC)^2$. Polydactyly entered the cross in association with the dominant alleles, L and N as in Table 15.

EXPERIMENT	ALLELES	DOMINANT				RECESSIVE				DEV./S.E.
		PERCENTAGE			TOTAL NO.	PERCENTAGE			TOTAL NO.	
		3-TOED	POOR 4-TOED	GOOD 4-TOED		3-TOED	POOR 4-TOED	GOOD 4-TOED		
$(CD)^2$	$C\ c^d$	71.0	21.0	8.0	224	66.7	33.3	0.0	63	0.7 N
$(CD)^2$	$F\ f$	71.4	21.7	6.9	203	66.7	28.0	5.3	75	0.8 N
$(CD)^2$	$P\ p$	74.1	20.3	5.6	197	61.1	31.1	7.8	90	2.2 N
$(BD)^2$	$S\ s$	75.3	21.3	3.4	291	67.7	24.7	7.5	93	1.4 N
$(BD)^2$	$C\ c^b$	72.9	23.6	3.5	288	75.0	17.7	7.3	96	0.4 L
$(BD)^2$	$P\ p$	72.4	22.9	4.8	293	76.9	19.8	3.3	91	0.9 L
$(BD)^2$	$B\ b$	75.2	19.8	5.0	298	67.4	30.2	2.3	86	1.4 N

rather large deviation from expectation also in the association of P,p with polydactyls in $(CD)^2$, but the direction is opposite to that expected for linkage. Again there is no significant corroboration from experiment $(BD)^2$. It is not necessary that strain D differ from the 3-toed strains C and B by the same genes, but it is probable that the major genes are the same.

These experiments involve normal polydactyly and ten of the 11 genes (including sex) whose relations have been considered in this paper. The gene not considered is *Px*, which determines monstrous polydactyly. The impossibility of distinguishing the effects of normal and monstrous polydactyly with certainty in many cases makes the study of linkage impracticable.

SUMMARY

Data are presented in which ten genes of the guinea pig (*S E A C F P B R M* and *Px*) are tested for possible linkage in every possible pair among themselves (in multiple crosses) and for possible partial sex linkage.

None of these genes exhibits either total or partial sex linkage.

One probable case of linkage was found. Genes *R* and *Px* gave recombination percentages of 41.10 ± 4.14 percent in coupling data in females, 39.29 ± 9.45 percent in repulsion data in females, 42.75 ± 3.01 percent in coupling data in males, 42.50 ± 5.59 percent in repulsion data in males, and in the grand total 42.08 ± 2.17 percent recombination or 3.65 times the standard error on the hypothesis of independence.

In no other case was there significant evidence of linkage. Excluding the case of *R* and *Px* and a few data in which classification was seriously doubtful, there was a total of 10,881 of the recombination classes in 21,867

or 49.76 percent recombination. Including all data, the average amount of recombination was 49.47 percent. Few organisms have been studied in which recombination occurs as freely as in the guinea pig. This is no doubt related to the very large number of chromosomes (65 in the male according to MOLS).

A multiple factor condition (occurrence of four toes in the hind feet instead of the normal three) was tested for possible linkage with all of the above genes except *Px* which also determines polydactyly. Other experiments have indicated that three or four major factors are responsible for normal polydactyly. The data suggest probable linkage of one of these with *A*. There are some other irregularities in the data but no clear indication of linkage with any of the others or with sex.

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BEARING ON HEREDITY AND VARIATION

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CORRIGENDA

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VOLUME 25

Page 541, first line in footnote, for "Valivov" read "Vavilov."

VOLUME 26

Page 40, line 34 (formula for Δ Q), for "W" in the denominator read "$\overline{W}$."
Page 45, line 3 (formula (20)), for "q" in the exponent of (1–q) read "$\overline{q}$."
Page 49, line 34, for "$\sigma_{\bar{q}}^{\bar{2}}$" read "$\sigma_{q}^{\bar{2}}$."
Page 49, line 36, for "$\sigma_{q}^{\bar{2}}$" read "$\sigma_{\bar{q}}^{\bar{2}}$."
Page 378, line 2, for "trisome" read "trisomic."
Page 383, line 18, for "table 1" read "table 2."
Page 526, line 5, for "third chromosome" read "fourth chromosome."

RAYMOND PEARL

RAYMOND PEARL was born at Farmington, New Hampshire, June 3, 1879, and died November 17, 1940, at Hershey, Pennsylvania.

PEARL graduated from Dartmouth College with the degree of A.B. in 1899. During his last year there he was assistant in the course in General Zoology which I was giving at the time. He showed even then the masterful and competent personality that marked him throughout life. He went with me to the University of Michigan in 1899, where for some years he was assistant in Zoology while at work as a graduate student. He received there in 1902 the degree of Doctor of Philosophy. From 1902 to 1906 he was Instructor in Zoology at the University of Michigan.

He married in 1903 MAUD M. DEWITT, herself a zoologist, who had held an important administrative place in the complex hierarchy of the large zoological laboratory of the University of Michigan. She cooperated extensively in his scientific work, and at the time of his death was Managing Editor of the journal "Human Biology" and assistant editor of the "Quarterly Review of Biology"—the two jorunals founded and edited by RAYMOND PEARL. With two daughters, RUTH and PENELOPE, MRS. PEARL survives him.

PEARL's dissertation was on the reactions and behavior of Planarians. Later he published a number of papers on genetical problems in lower organisms. He then decided to enter the field of the application of statistical methods to biological problems and spent the year 1905–1906 at work with KARL PEARSON in London. He there presented a paper before the Royal Society and became in 1906 Associate Editor (with PEARSON) of "Biometrika." But it was difficult for two such strong and aggressive personalities as PEARSON and PEARL to work together; there was a falling out, and the connection with "Biometrika" ceased in 1910. PEARL returned to America in 1906 and was Instructor in Zoology at the University of Pennsylvania in 1906–1907. His contributions to biology were now mainly in the statistical field. In 1907 he went to the University of Maine as biologist and head of the Department of Biology of the Maine Agricultural Experiment Station, remaining there until 1918. During this period he made numerous contributions on the genetics and biology of the fowl and of other domestic animals, many of these published with his associate F. M. SURFACE. He published also a work on the diseases of fowls (1911) and a critique on "The Modes of Research in Genetics" (1913). His work in this period showed a mastery of statistical methods such that on the entrance of America into the first world war he was called to Washington by HOOVER to act as Chief of the Statistical Division of the United States Food Administration (1917–1919). He built up a large organization, which

included a number of well-known men of science, and published a book on "The Nation's Food"; also articles on biological aspects of war. At the end of the war, at the instance of DR. WILLIAM WELCH, PEARL was called to become Professor of Biometry and Vital Statistics in the newly founded School of Hygiene and Public Health of the Johns Hopkins University. He organized a department in this field, with LOWELL J. REED as Associate Professor, and gave courses in statistical methods and their applications in medicine and biology. A product of this period is his well known textbook entitled "Introduction to Medical Biometry and Statistics," published in 1923; a third edition of this has recently appeared. As statistician of the Johns Hopkins Hospital, he systematized autopsy records and published at intervals data or conclusions based upon study of these. From this time his work was mainly devoted directly or indirectly to the biology of man, though he employed other organisms for experimental purposes. He published in 1922 a volume on "The Biology of Death," based on his Lowell Lectures given in 1920. From 1922 to 1927 appeared his "Experimental Studies on the Duration of Life," based on work on Drosophila. The results of these studies, with much other material, were embodied in his books on "The Biology of Population Growth" (1925) and on "The Rate of Living" (1928). An extensive statistical investigation of the effects of the use of alcohol on longevity and mortality in man was published as a book under the title "Alcohol and Longevity" in 1926. This investigation led him to the conclusion that the moderate consumption of alcohol is not harmful. These studies and conclusion were widely publicized. A similar study made in later years on the effects of the use of tobacco led him to the conclusion that tobacco is harmful even in small quantities; this again attracted much attention in the press.

From 1920 on appeared a series of papers with L. J. REED on Population Growth and Its Mathematical Representation, culminating in a curve of population growth, which was employed for forecasting the course of population growth in later periods. This, like much of PEARL's work, aroused interest and controversy.

Other contributions to the biology of man dealt with the biological nature and classification of diseases and with biological aspects of certain special diseases, notably tuberculosis, cancer, and influenza. Many contributions dealt with human reproduction, including a series of reports on the problems and results of birth control. A volume of his collected papers on the biology of man, entitled "Studies in Human Biology," was published in 1924.

In 1923 PEARL received the title of Professor of Biology in the Medical School. In 1925 he gave up the direction of the work in the statistical laboratory and was succeeded by LOWELL J. REED. In that year PEARL became

Director of the Institute for Biological Research, an enterprise maintained in connection with the Johns Hopkins University for five years by the Rockefeller Foundation. During this period he was Research Professor in the University. At the end of the five year period, in 1930, he became Professor of Biology in the School of Hygiene and Public Health, remaining in this position until his death.

In 1926 PEARL founded the "Quarterly Review of Biology." In addition to general articles by authorities, reviewing the situation in particular fields of biology, and lists of important new publications, this journal included a department of comments and reviews on recent biological literature by PEARL himself—a department that has been much appreciated. In 1929 he founded the journal "Human Biology"; for the publication of detailed investigations on the biology of man. He directed or edited these two journals until the time of his death.

The two main directions of PEARL's activities—on the one hand in biological investigations, on the other hand in the field of statistical research—had each brought him many honors. In the former field, he had been President of the American Society of Zoologists (in 1913) and of the American Society of Naturalists (1916–17). In the latter field he had been President of the American Statistical Association, of the American Association of Physical Anthropologists, and of the International Union for the Scientific Investigation of Population Problems. He was a member of the National Academy of Sciences, of the American Philosophical Society, and of the American Academy of Arts and Sciences. He was decorated as Knight (later Officer) of the Crown of Italy.

As is perhaps indicated in even so brief a summary, PEARL was a man of masterful personality, of extraordinary resourcefulness and initiative, of wide knowledge, astonishing power of work, and remarkable versatility. He was active in many directions outside the fields of his technical work. He was socially prominent and popular, of wide acquaintance in America and England, and numbered among his friends some of the most interesting personalities of his time. He was a musical amateur of some attainment. He belonged in Baltimore to a group of choice spirits centering about H. L. MENCKEN which, under the name of the Saturday Evening Club, met for social and musical purposes. To the members of this Club he dedicated his volume on "Alcohol and Longevity." He published in 1927 a small volume entitled "To Begin With, Being Prophylaxis against Pedantry" in which he set forth characteristic views as to the background of reading appropriate to the student of science or to the man of the world. Disillusionment and sophistication were the objects sought for; such satirical writings as those of LUCIAN, RABELAIS, and VOLTAIRE were highly recommended. A medical colleague characterized PEARL as a rare embodiment of

the Pantagruelesque ideal of enjoying to the full all the activities and pleasures of life—including certainly in his case the very great pleasures of work and of accomplishment in many difficult fields.

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COLIN, EDWARD C. *Elements of Genetics.* Pp. xii+386. Figs. 47. The Blakiston Co. Philadelphia, 1941. $3.

DEMEREC, M., and eleven others. *Cytology, genetics, and evolution.* [Papers given at the University of Pennsylvania Bicentennial Conference.] Pp. v+168. Figs. numbered separately for each paper. University of Pennsylvania Press. Philadelphia. 1941. $2.00.

FELT, EPHRAIM PORTER. Pruning trees and shrubs. Pp. 237. Figs. 94. Orange Judd Pub. Co. New York, 1941. $2.00.

POOL, RAYMOND J. *Flowers and flowering plants.* Second Edition. Pp. xxiii+428. Fig. 210. McGraw-Hill Book Co. New York, 1941. $3.50.

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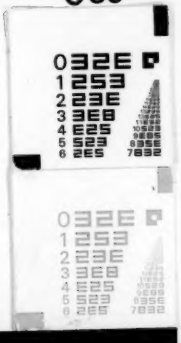
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